University of Alberta

The synthesis of exo-peptides and chiral diamine ligands

Dehe Li ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

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Dec. 06, 2004

Abstract

Two projects, the synthesis of exo-peptides (acetylated polyamines) and the synthesis of chiral diamines, were discussed in this thesis. In the exo-peptide project, no linker containing a C-O ether attachment to resins was found to be resistant to borane-tetrahydrofuran reduction conditions. Also, aggregation occurred during peptide bond extension onto solid support, and the low solubility of model polyalanine peptides caused problems during the formation of peptide bonds in solution phase. In the chiral diamine project, the diamine precursors, chiral polyamides, were constructed on solid support followed by borane-tetrahydrofuran reduction and functionalization. Four different chiral diamines, including one linear secondary, one linear tertiary, one six-membered and one seven-membered tertiary diamines, were synthesized. Although no enantiomeric excess value was observed when these diamines were tested for two asymmetric reactions, it is clear that a large variety of chiral diamines could be synthesized using this general method and tested for asymmetric reactions in the future.

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Table of Contents

Chapter 1 Introduction – Unnatural Biopolymers 1

1.1 Definition of peptides 1
1.2 Biological activities of peptides and other unnatural biopolymers 1
1.3 Secondary structures of peptides and other unnatural biopolymers 4
1.4 Bibliography 9

Chapter 2 The Synthesis of Exo-peptides 11

2.1 The background of the synthesis of peptides and polyamines 11
2.1.1 Peptide bond formation in solution phase 11
2.1.2 Strategies for forming long peptides 15
2.1.3 Synthesis of peptides on solid-phase 16
2.1.4 Synthesis of polyamines 18
2.2 Perspective and objective of the project 24
2.3 The synthesis of exo-peptides 27
2.3.1 The synthesis of exo-peptides through solid phase chemistry 27
2.3.1.1 Mitsunobu strategy using C-O bonds as resin anchors 27
2.3.1.2 Mitsunobu strategy using C-N bonds as resin anchors 40
2.3.1.3 Fragment coupling strategy for the synthesis of exo-peptides 44
2.3.1.4 Stepwise approach for the synthesis of exo-peptides 49
2.3.2 The synthesis of exo-peptides through solution phase 53
2.3.2.1 Synthesis of dipeptide **90** 55
2.3.2.2 Synthesis of tripeptide **93** 56
2.3.2.3 Synthesis of tetrapeptide **95** 58
2.3.2.4 Model study of acetyl pentaamine 60
2.3.2.5 Synthesis of octapeptide **137** 62
2.4 Summary 65
2.5 Experimental Section 66
2.5.1 General 66

2.5.2 Synthesis of starting materials 69
2.5.3 Loading Fmoc-alaninol to resin 71
2.5.4 Synthesis of trityl resin bound Dde protected triamine **24** 75
2.5.5 Synthesis of Boc protected polyamines **65** 77
2.5.6 Synthesis of peptide **66** 78
2.5.7 Synthesis of resin bound peptide **75** 78
2.5.8 Synthesis of peptide **77** 79
2.5.9 Synthesis of dipeptide **90** 80
2.5.10 Synthesis of tripeptide Fmoc-$(Ala)_3$-O*t*-Bu (**93**) 81
2.5.11 Synthesis of Fmoc-$(Ala)_4$-O*t*-Bu (**95**) 82
2.5.12 Synthesis of Synthesis of octapeptide **106** 83
2.6 Biliography 85

Chapter 3 The Synthesis of Chiral Diamines 89

3.1 Introduction 89
3.2 Perspective and objective of the project 93
3.3 Synthesis of chiral diamines 95
3.3.1 Synthesis of resin bound piperidine linker **129** and diamine **135** 96
3.3.2 Synthesis of diamine **130** 98
3.3.3 Synthesis of diamine **147** 101
3.3.4 Synthesis of diamine **148** and diamine **149** 103
3.4 Preliminary reactions screens using chiral diamines 105
3.4.1 Catalytic asymmetric allylation of hydrazono esters 105
3.4.2 Enantioselective palladium (II)-catalyzed aerobic alcohol oxidation .. 106
3.4.3 Copper-catalyzed coupling of amides with a vinyl bromide 107
3.5 Summary 108
3.6 Experimental section 109
3.6.1 General 109
3.6.2 Synthesis of 4-(Dde-aminomethyl)piperidine **133** 109
3.6.3 Synthesis of resin **134** 109

3.6.4 Synthesis of resin **132** 110
3.6.5 Synthesis of resin bound diamine **135** 111
3.6.6 Synthesis of **139** 111
3.6.7 Synthesis of **140** 112
3.6.8 Synthesis of diamine **130** 112
3.6.9 Synthesis of compound **145** 114
3.6.10 Synthesis of tertiary diamine **147** 114
3.6.11 Synthesis of cyclic tertiary diamine **148** 115
3.6.12 Synthesis of cyclic tertiary diamine **149** 116
3.7 Bibliography 118

Chapter 4 General Conclusions 120

4.1 Summary, conclusions, and future directions with exo-peptides 120
4.2 Summary, conclusions, and future directions with chiral diamines 123

Appendix of selected spectra 124

List of Tables

Table 2.1 Loading of Fmoc-alaninol to trityl chloride resin.....30
Table 2.2 BH_3-THF reduction of resin bound peptide **17** 32
Table 2.3 Reduction of peptides on various resins.....40
Table 2.4 Protecting polyamines with Nbs, trityl and Boc groups 48

List of Figures

Figure 1.1 Generic structure of peptides....1

Figure 1.2 Structure of peptide-penicillin conjugate **1**....2

Figure 1.3 Generic structures of oligocarbamates **2**....2

Figure 1.4 Structures of polyamines and polyamine analogues....3

Figure 1.5 Structure of β-peptide **10** used by Seebach and co-workers....5

Figure 1.6 Structures of β-sheets proposed by Schreiber and co-workers
A: antiparallel β-sheet B: parallel β-sheet....5

Figure 1.7 Structures of 3,5-linked polypyrrolinones used by the Smith group....6

Figure 1.8 Structures of β-peptides synthesized by Gellman and coworkers
A: Tetramer and hexamer of *trans*-2-aminocyclohexane carboxylic acid;
B: Hexamer and octamer of *trans*-2-aminocyclopentane carboxylic acid....6

Figure 1.9 Oligomeric *N*-substituted glycines....7

Figure 1.10 Structure of oligoanthranilamide **13**....7

Figure 1.11 Structure of vinylogous aminosulfonic dipeptide **14**....8

Figure 2.1 Coupling reagents for peptide amide bond formation....15

Figure 2.2 Structures of exo-peptides....25

Figure 2.3 Conformational analysis of exo-peptides....25

Figure 2.4 Hypothetical mechanism of release of compounds from Wang resin....34

Figure 2.5 Protected amino alcohols used as Mitsunobu reaction substrates....43

Figure 3.1 Chiral diamines and chiral diamine derivatives used as ligands....90

Figure 3.2 The structures of diamine **1** and its *ortho*-palladated complex **121**....91

Figure 3.3 Generic structure of diamines....94

Figure 3.4 The structures of diamines **148** and **149**....103

Figure 4.1 General structures of chiral diamines....123

List of Schemes

Scheme 2.1 General activating strategy for peptide bond formation 11

Scheme 2.2 Peptide bond formation through mixed anhydride intermediates 12

Scheme 2.3 Peptide bond formation through nitrophenyl esters 12

Scheme 2.4 Peptide bond formation using the coupling reagent DCC 13

Scheme 2.5 Racemization of peptides through oxazolones 14

Scheme 2.6 Peptide bond formation using DCC/HOBt 14

Scheme 2.7 Peptide bond formation by stepwise strategy 16

Scheme 2.8 Peptide bond formation by segment coupling strategy 16

Scheme 2.9 General procedure of peptide synthesis on solid support 17

Scheme 2.10 Synthesis of polyamine **6** by Edwards and co-workers 18

Scheme 2.11 Synthesis of polyamine **7** by Houghten and co-workers 19

Scheme 2.12 Synthesis of polyamine **9** and acetylated polyamine **10** by Hall and co-workers 20

Scheme 2.13 Synthesis of PhTX-433 (**11**) by Hall and co-workers 21

Scheme 2.14 Synthesis of PhTX-433 (**11**) by Bycroft and co-workers 22

Scheme 2.15 Synthesis of PhTX-433 (**11**) by Jaroszewski and co-workers 23

Scheme 2.16 Synthesis of triazacycloalkane **12** by Hall and co-workers 24

Scheme 2.17 Retrosynthetic approach to exo-peptides 26

Scheme 2.18 Synthetic approach using a Mitsunobu coupling strategy 28

Scheme 2.19 Synthesis of resin bound Fmoc-alaninol (**16**) 29

Scheme 2.20 Synthesis of resin bound triamines **18**, **24** and **25** 31

Scheme 2.21 Construction of Dde protected triamine on Wang resin 33

Scheme 2.22 4-Hydroxylmethylphenyl methylamine as a linker 34

Scheme 2.23 Hypothetic mechanism of the loading step of resin **31** in THF 36

Scheme 2.24 Hypothetic mechanism of the loading step of resin **31** in DCM 36

Scheme 2.25 Attempted preparation of Dde protected triamine onto DHP resin 37

Scheme 2.26 Attempted preparation of Dde protected triamine onto diphenyldiazomethane resin 38

Scheme 2.27 Attempted preparation of Dde protected triamine onto silyl resin 39

Scheme 2.28 C-N bonds as linkers for Mitsunobu strategy 41
Scheme 2.29 Preparation of resin **53** by re-attaching **52** to resin 41
Scheme 2.30 Evaluation of re-attachment step by Dde protected amine **55** 42
Scheme 2.31 Preparation of resin **57**, a Mitsunobu reaction substrate 43
Scheme 2.32 Free amino alcohol used as a Mitsunobu reaction substrate by the Bradley group 44
Scheme 2.33 Alcohols used as substrates under Mitsunobu conditions 44
Scheme 2.34 Fragment coupling strategy for the synthesis of exo-peptides 45
Scheme 2.35 Protection of resin bound polyamine **62** and **64** by Nbs group 46
Scheme 2.36 Protection of resin bound amine **62** using the Boc group 47
Scheme 2.37 Indirect method for purity check of trityl protected polyamines 49
Scheme 2.38 Plans for stepwise approach for the synthesis of exo-peptides 50
Scheme 2.39 Preparation of acetylated triamine **79** 50
Scheme 2.40 General stepwise procedure for the synthesis of resin bound peptides 51
Scheme 2.41 The use of Hmb amino acids in the synthesis of peptides 52
Scheme 2.42 General segment approach for peptide synthesis in solution phase 54
Scheme 2.43 Fragment approach of synthesis of polyalanine peptides by the Cameron group 55
Scheme 2.44 Preparation of Fmoc-Ala-Ala-O*t*-Bu 55
Scheme 2.45 Initial plan for the synthesis of Fmoc-$(Ala)_4$-O*t*-Bu 56
Scheme 2.46 Selective deprotection of Fmoc or *t*-Bu groups 57
Scheme 2.47 Stepwise approach for the synthesis of Fmoc-$(Ala)_4$-O*t*-Bu 58
Scheme 2.48 Preparation of Fmoc-$(Ala)_3$-O*t*-Bu tripeptide 58
Scheme 2.49 Preparation of tetrapeptide **95** using DCC/HOBt 59
Scheme 2.50 Preparation of tetrapeptide **95** through mixed anhydride intermediate **96** 60
Scheme 2.51 Preparation of short exo-peptide **103** 61
Scheme 2.52 Preparation of octapeptide **106** 62
Scheme 2.53 Preparation of polyamine **109** 64
Scheme 2.54 Alternative route for the preparation of peptide **108** 65
Scheme 3.1 The synthesis of chiral diamine **122** by the Shono group 91

Scheme 3.2 The synthesis of chiral diamine **123** by the Burrows group 92
Scheme 3.3 The synthesis of chiral diamine **124** by the Salvadori group 92
Scheme 3.4 The synthesis of chiral diamine **125** by the Fujisawa group 93
Scheme 3.5 Retrosynthetic approach to chiral diamines 95
Scheme 3.6 Retrosynthetic approach for diamine **130** 96
Scheme 3.7 Preparation of resin bound piperidine linker **129** 96
Scheme 3.8 Preparation of resin bound diamine **135** 97
Scheme 3.9 Protection of resin bound diamine **135** with Teoc group 99
Scheme 3.10 Preparation of diamine **130** 100
Scheme 3.11 Benzylation of resin bound diamine **135** with benzyl bromide 102
Scheme 3.12 Benzylation of resin bound diamine **143** with benzyl bromide 102
Scheme 3.13 Preparation of diamine **147** 103
Scheme 3.14 Preparation of diamine **148** 104
Scheme 3.15 Preparation of diamine **149** 105
Scheme 3.16 Catalytic asymmetric allylation of hydrazono ester **150** 106
Scheme 3.17 Enantioselective palladium (II)-catalyzed aerobic alcohol oxidation 107
Scheme 3.18 Copper-catalyzed coupling of amide **153** with vinyl bromide **155** 107
Scheme 3.19 Possible enantioselective vinylation of 2-pyrrolidinone derivatives 108
Scheme 4.1 Possible approach to prepare resin bound polyamines 121
Scheme 4.2 Possible approach to prepare compound **157** on solid support 122
Scheme 4.3 Possible approach to prepare compound **157** in solution phase 122

List of abbreviations

δ	chemical shift downfield from 0 ppm
^{13}C NMR	carbon-13 nuclear magnetic resonance
^{1}H NMR	proton nuclear magnetic resonance
9-BBN	9-borabicyclo[3.3.1]nonane
Ac	acetyl
Ac_2O	acetic anhydride
AcOH	acetic acid
ADDP	1,1'-(azodicarbonyl)dipiperidine
Ala	alanine
app.	apparent
APT	attached proton test
Bn	benzyl
BOC	*tert*-butoxylcarbonyl
Boc	*t*-butoxycarbonyl
BOP	benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate
BOP-Cl	bis(2-oxo-3-oxazolidinyl)phosphinic chloride
Bz	benzoyl
calcd.	calculated
CIC	1-cyclohexyl-3-isopropyl carbodiimide
d	doublet
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	dicyclohexylcarbodiimide
DCE	dichloroethane
DCM	dichloromethane
dd	doublet of doublets
Dde	1-(4,4-dimetyl-2,6-dicyclohexylidene)ethyl
Dde-OH	2-acetyldimedone
DEAD	diethylazodicarboxylate

DEAD	diethylazodicarboxylate
DHP	dihydropyran
DHP resin	3,4-digydro-2H-pyran-2-ylmethoxymethyl polystyrene
DIAD	diisopropyl azodicarboxylate
DIC	1,3-diisopropyl carbodiimide
DIPEA	*N,N*-diisopropylethylamine
DMAP	*N,N*-dimethyl-4-amino pyridine
DMF	*N,N*-dimethylformamide
equiv.	equivalents
ES MS	electrospray mass spectrometry
Et	ethyl
Fmoc	9-fluorenylmethoxycarbonyl
g	grams
gCOSY	gradient-selected correlation spectroscopy
HATU	2-(7-aza-1H-benzotriazole-1-yl)-1,1,3,,3-tetramethyluronium hexafluorophosphate
HBTU	*O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyluronium hexafluorophosphate
HBTU	benzotriazo-1-yl-*N*-tetramethyl-uronium hexafluorophosphate
HIV	human immunodeficiency virus
Hmb	2-hydroxy-4-methoxybenzyl
HMBC	heteronuclear multiple bond coherence
HMQC	heteronuclear multiple quantum coherence
HOAT	7-aza-1-hydroxybenzotriazole
HOBt	1-hydroxybenzotriazole
HPLC	high performance liquid chromatography
HR MS	high resolution mass spectrometry
Hz	hertz
i-pr	*iso*-propyl
IR	infrared
LC-MS	liquid chromatography-mass spectrometry
LC-UV	liquid chromatography-ultraviolet spectrophotometry

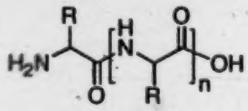
m	multiplet
M	molar
m/z	mass to charge ratio
m-CPBA	*m*-chloroperoxybenzoic acid
Me	methyl
MeCN	acetonitrile
mg	milligrams
min	minutes
mL	milliliters
mmol	millimoles
MS	mass spectrometry
Nbs	*o*-nitrobenzenesulfonyl
°C	degrees Celsius
Ph	phenyl
PhTX	philanthotoxin
PMP	1,2,2,6,6-pentamethyl piperidine
ppm	parts per million
PPTS	pyridium *p*-toluenesulfonate
PTSA	*p*-toluene sulfonic acid
PyBOP	benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate
q	quartet
quint.	quintet
R_f	retention factor
RNA	ribonucleic acid
Rt	retention time
s	singlet
Su	succinimidyl
t	triplet
TBAF	tetrabutyl ammonium fluoride
TBP	tributylphosphine

t-Bu	*tert*-butyl
Teoc-	2-(trimethylsilyl)-ethoxycarbonyl
Tf_2O	trifluoromethanesulfonic anhydride
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TPP	triphenylphosphine
Trt	trityl
TrtCl	tritylchloride
UV	ultraviolet

Chapter 1 Introduction – Unnatural Biopolymers

1.1 Definition of peptides

Peptides, polyamines and proteins play key roles in biology. According to the IUPAC definition, peptides are "amides derived from two or more amino carboxylic acid molecules (the same or different) by formation of a covalent bond from the carbonyl carbon of one to the nitrogen atom of another with formal loss of water. The term is usually applied to structures formed from α-amino acids, but it includes those derived from any amino carboxylic acid."[1] (Figure 1.1)

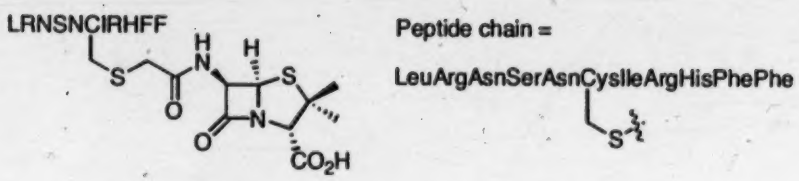


"(R may be any organyl group, commonly but not necessarily one found in natural amino acids)"

Figure 1.1 Generic structure of peptides

1.2 Biological activities of peptides and other unnatural biopolymers

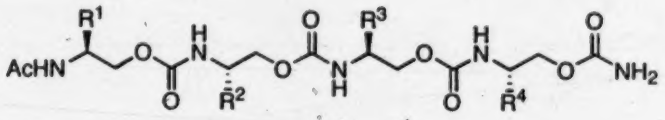
Peptides are widely used to investigate biological functions, such as enzymes kinetic mechanism of action, biochemical and physiological roles. They are also used in the application for the isolation of enzymes, and in the design of inhibitors. For example, two 36-residue peptides, $N36^{Mut(e,g)}$ and $N36^{Mut(a,d)}$, were used as inhibitors of HIV fusion, which disrupt the internal trimeric coiled-coil of gp41.[2] Some peptides have already found use as therapeutic agents. For example, calcitonin has been used as therapy of acute cancer, related hypercalcemia in children.[3] The Roberts group stated that a peptide-penicillin conjugate **1** (Figure 1.2) showed a 100-fold higher activity than the parent penicillin towards the *Staphylococcus aureus* penicillin binding protein 2a (PBP2a).[4] The PBP2a protein is responsible for the resistance of *Staphylococcus aureus* strain to β-lactam drugs.



1

Figure 1.2 Structure of peptide-penicillin conjugate **1**

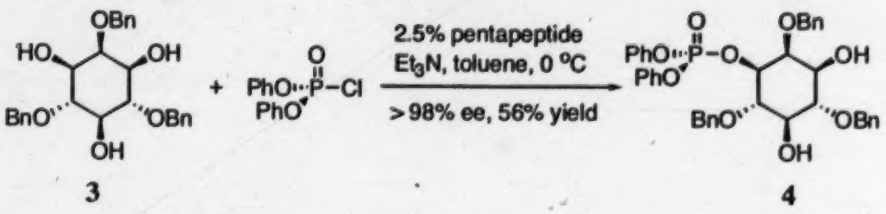
Other unnatural biopolymers have also shown biological activities. For example, Schultz and co-workers reported that oligocarbamates **2**, such as $AcY^cK^cF^cL^cG$-OH (the superscript "c" indicates the presence of a carbamate linkage) and $AcY^cI^cF^cL^cG$-OH (Figure 1.3), show strong binding abilities to a monoclonal antibody (mAb), α-$AcY^cK^cF^cL^c$mAb.[5] These highly hydrophobic oligomers are more resistant to proteolytic degradation than their corresponding peptides. The Rana group [6] successfully assembled an 11-unit polypeptoid (oligomeric *N*-substituted glycine), which showed strong affinities towards the ***trans***-activation responsive region (TAR) RNA, a 59-base stem-loop structure located at the 5'-end of all HIV mRNAs.



2

Figure 1.3 Generic structures of oligocarbamates **2**

In addition to their applications in biology and biochemistry, peptides can also be used as catalysts in chemical reactions. Very recently, Miller described some elegant enantioselective phosphorylation reactions using peptides as catalysts (Equation 1).[7]



(1)

Like peptides, naturally occurring polyamines, such as putrescine (**5**), spermidine (**6**) and spermine (**7**) (Figure 1.4), which are ubiquitous compounds in microorganisms, plants and animals, also have strong biological activities. Polyamines form strong interactions with DNA and render DNA relatively stable to heat, pH variation and radiation exposure.[8,9] The concentration of polyamines (spermine, spermidine, putrescine and cadaverine) decreases with improvement in the patient's condition in multiple therapy, and could thus be used as cancer markers[10]. Monitoring this change can be used for evaluating the success of a given therapy. Like polyamines, their analogues also display significant biological activities and represent potential drug candidates. Studies *in vivo* and *in vitro* indicated that the alkylated polyamine BENSpm (**8**) (Figure 1.4) is a very promising antitumor agent.[11] In addition, polyamine analogue MDL 27695 (**9**) (Figure 1.4) has been used as antimalarial agent.

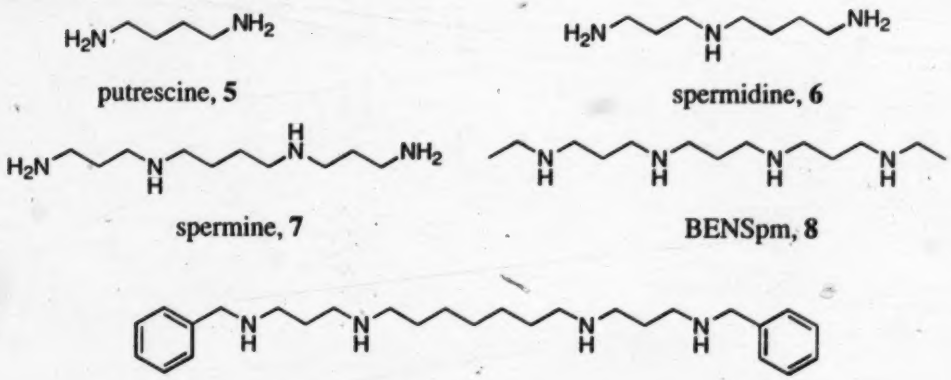
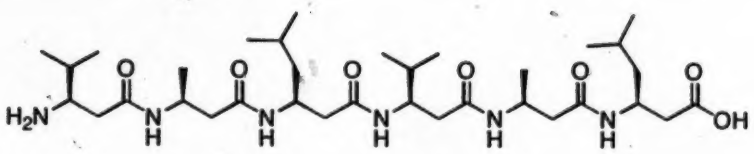


Figure 1.4 Structures of polyamines and polyamine analogues

1.3 Secondary structures of peptides and other unnatural biopolymers

Proteins show secondary structures, typically alpha helices or beta sheets, that are subdivided into parallel and antiparallel sheets.[12] Helical structures are stabilized by intra-chain hydrogen bonds, whereas in beta sheets inter-chain hydrogen bonds occur between the carbonyl oxygen and the amide NHs of every other residue in the backbone. Due to these types of secondary structures, proteins, DNA, and other biological molecules can fold in a specific orientation. They adopt well-ordered conformations, in a way such that their active sites bind or interact with other biological polymers (such as enzymes), allowing sophisticated reactions to take place under mild conditions. For a long time, chemists and biologists alike have tried to understand these unique properties and mimic them for research or therapy purposes.[13] The tools developed encompass the synthesis and study of DNA and protein analogues.

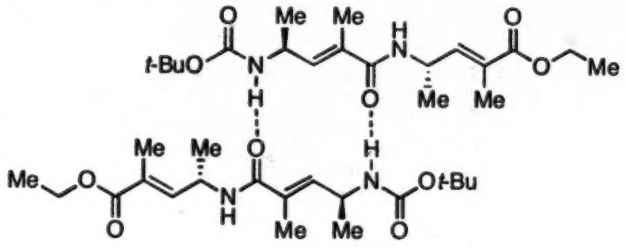
Before designing peptides and analogues thereof possessing secondary structures, one should first understand the general rules governing the formation of secondary structures. In Gellman's review[14], it was pointed out that the main concepts of designing polymers which have strong tendency to adopt specific compact conformation are the backbone of the polymers and the features of the repetitive units. Proper backbones, such as appropriate stereogenic centers within backbones, will be helpful for secondary structure formation. Due to steric effects, suitable features of repetitive units can reduce steric hindrance and minimize the energy of resulting secondary conformations as a result of by hydrogen bond formations. For example, Toniolo and co-workers[15] prepared terminally blocked homopeptides Y-[L-(αMe)Val]$_n$-O*t*-Bu (Y= benzyloxycarbonyl, *para*-bromobenzoyl, Ac, n = 2-8) and found that the octamer attained a stable, fully populated right-handed 3_{10}-helical structure. A high percentage of 3_{10}-helix was observed from pentamer to heptamer. β-Peptides with proper backbones can form secondary structures. For example, Seebach and co-workers reported that β-peptide **10** adopts a 14-helical conformation in pyridine-d_5 and methanol (Figure 1.5).[16, 17]



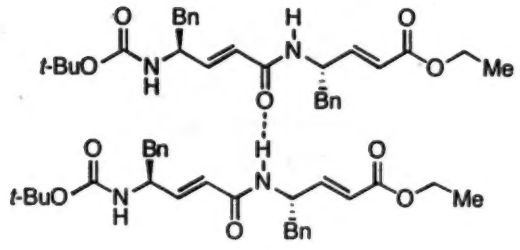
10

Figure 1.5 Structure of β-peptide **10** used by Seebach and co-workers

Schreiber and co-workers have attempted to analyze the secondary and tertiary structures of vinylogous peptides.[18] From crystallographic analysis, it was revealed that the vinylogous dipeptide bearing methyl groups adopted a secondary structure featuring an antiparallel β-sheet (Figure 1.6 A), whereas the benzyl bearing counterpart adopted a parallel β-sheet secondary structure (Figure 1.6 B).



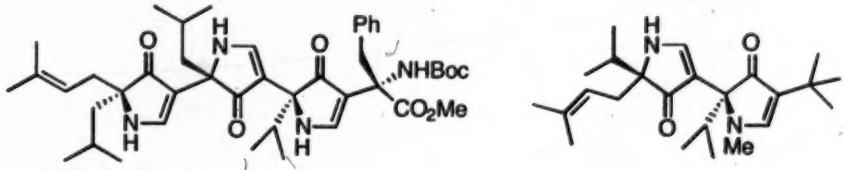
A



B

Figure 1.6 Structures of β-sheets proposed by Schreiber and co-workers A: antiparallel β-sheet B: parallel β-sheet

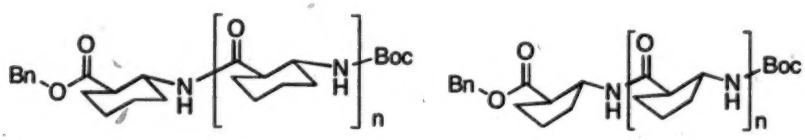
The Smith group reported that 3,5-linked trispyrrolinone **11** adopted antiparallel β-sheets and the β-strand conformation in the solid state, due to intramolecular and interstrand hydrogen bonding.[19, 20] Interestingly, methylated bispyrrolinone **12** formed a helix in the solid state (Figure 1.7).



11 **12**

Figure 1.7 Structures of 3,5-linked polypyrrolinones used by the Smith group

The Gellman group applied computational methods [21] to evaluate 72 hypothetical possible β-peptide helix structures with a rigid 6-membered ring, and predicted that the most stable helical structure would be the 14-helical form of the decamer of *trans*-2-aminocyclohexane carboxylic acid. The prediction was confirmed by X-ray crystallographic analysis of the tetramer (n = 3) and the hexamer (n = 5) and ^{1}H-NMR spectra (Figure 1.8 A). A hexamer (n = 5) and an octamer (n = 7) of *trans*-2-amino-cyclopentane carboxylic acid (Figure1.8 B) have also shown 12-helical conformations.[22]



A (n = 3, 5) **B** (n = 5, 7)

Figure 1.8 Structures of β-peptides synthesized by Gellman and coworkers
A: Tetramer and hexamer of *trans*-2-aminocyclohexane carboxylic acid; B: Hexamer and octamer of *trans*-2-aminocyclopentane carboxylic acid

Barron and co-workers pointed out that peptoids (oligomeric *N*-substituted glycines) with α-chiral, aromatic side chains show stable helix in organic and aqueous

solution, despite lacking backbone chirality and unfavorable formation of intrachain hydrogen bonds (Figure1.9).[23, 24]

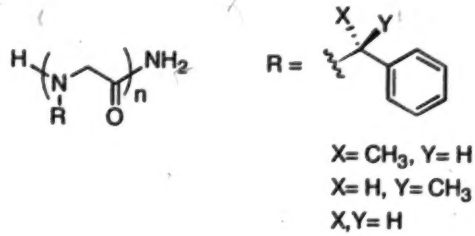
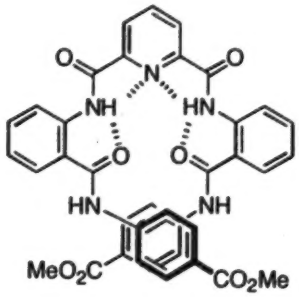


Figure 1.9 Oligomeric *N*-substituted glycines

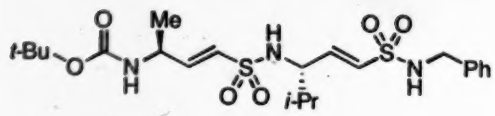
Hamilton and co-workers reported that oligoanthranilamide **13** formed a helical conformation in the solid state. This behavior is likely due to the intramolecular interactions, the intrinsic rigidity of the molecule and the *trans* preference of the secondary amide bonds (Figure 1.10).[25]



13

Figure 1.10 Structure of oligoanthranilamide **13**

Vinylogous sulfonopeptides were synthesized and examined by the Gennari group.[26, 27] It was found that twelve-membered-ring hydrogen bond was formed in the crystal structure of chiral vinylogous aminosulfonic dipeptide **14**, while fourteen-membered-ring hydrogen bonding was the most common folding pattern for the oligomers in chloroform solution (Figure 1.11).



14

Figure 1.11 Structure of vinylogous aminosulfonic dipeptide **14**

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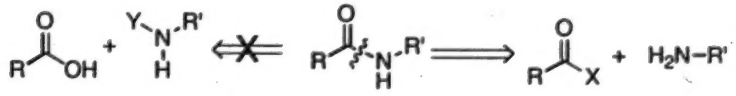
Chapter 2 The Synthesis of Exo-peptides

2.1 The background of the synthesis of peptides and polyamines

Since Emil Fischer introduced the concept of peptides in the early 1900s, various methods for peptide bond formation have been developed both in solution phase and solid phase. Solution phase methods are time consuming, and not productive enough to meet drug discovery requirements. Since Merrifield introduced the solid phase technique in the early 1960's, peptide synthesis methods on solid phase have been developed dramatically and have been extensively applied. [1]

Carboxylic acids and amines do not form amide bonds spontaneously. Theoretically, either the acid or the amine needs to be activated (Scheme 2.1), but up to now peptide bond formation still relies on activation of carboxylic acid moieties.[2]

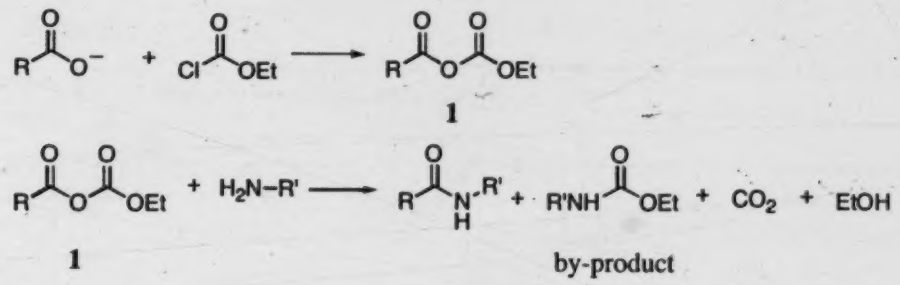


Scheme 2.1 General activating strategy for peptide bond formation

2.1.1 Peptide bond formation in solution phase

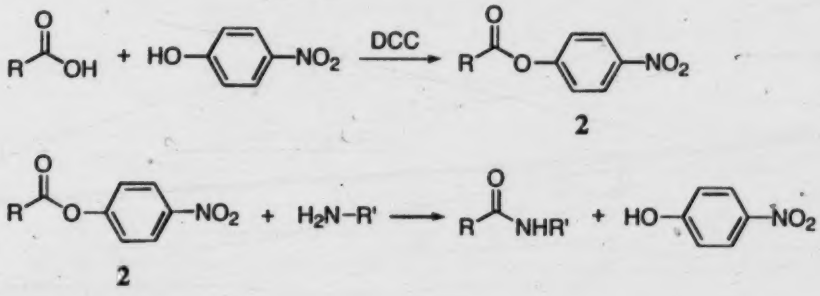
To generate peptide amide bonds, at least three issues must be considered:[2, 3] 1) reactivity of reagents; 2) protection and deprotection strategies; 3) racemization. As mentioned above, in order to couple with amine species, acid moieties must be activated. Methods to activate carboxylic acid have been well described and they include trans-formation to more reactive acid chlorides, acid azides, mixed anhydrides, active esters and the use of coupling reagents. The first two methods involving acid chlorides and azides are too reactive and suffer side reactions and racemization. For these reasons, the latter methods mentioned above have become important. In the anhydride method,[4] for example, a carboxylic acid reacts with ethyl chlorocarbonate to generate a mixed anhydride intermediate **1** (Scheme 2.2). In the intermediate **1**, because an ethoxy group has stronger electron donating ability than an alkyl group, the carbonyl group of the

activated carboxy-component has less electronic density; is more electrophilic, and therefore preferentially attacked by the amine. There is minimal amount of by-product, formed via attack of the carbonic acid carbonyl group, which can be further reduced when isobutyl chlorocarbonate is used. Easy work-up is an attractive feature of this reaction because only carbon dioxide, ethanol and small amount of by-products are formed.



Scheme 2.2 Peptide bond formation through mixed anhydride intermediates

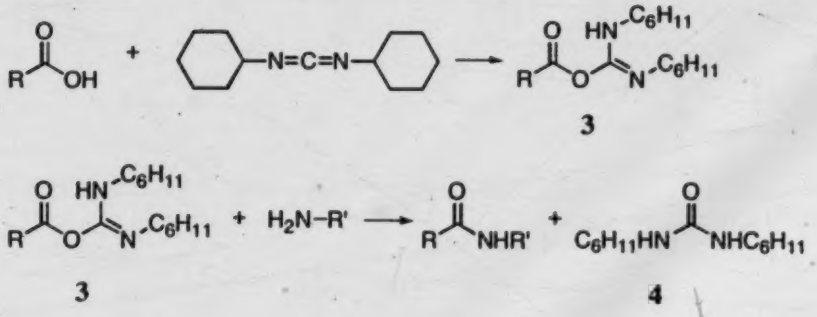
To avoid "over activation" of carboxylic acids, the active ester approach was introduced. Carboxyl components are first converted to nitrophenyl esters **2**, which undergo nucleophilic attack by amines to give peptides (Scheme 2.3).



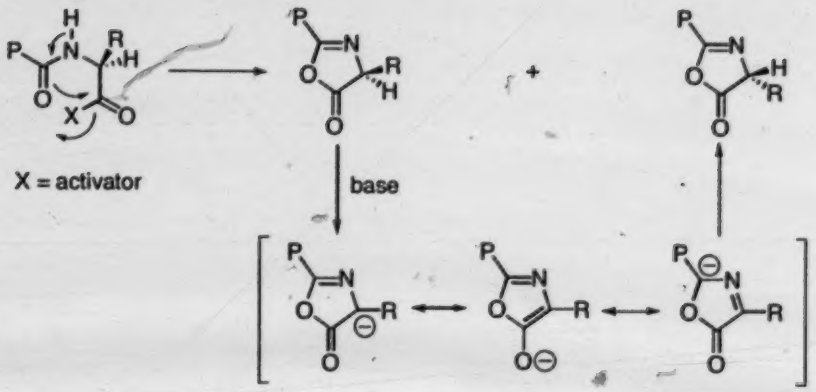
Scheme 2.3 Peptide bond formation through nitrophenyl esters

The advantage of this method lies in the stability of the nitrophenyl esters **2**, which can be separated, characterized and stored for years. The slow aminolysis step can be catalyzed by adding 1-hydroxybenzotriazole (HOBt).[5,6]

Since the discovery of dicyclohexylcarbodiimide (DCC) in 1955, coupling reagents have continuously gained importance in peptide synthesis.[7] This "one pot" reaction was the chemists' dream for forming peptide bonds. By simply adding DCC to a mixture of an acid and an amine, DCC almost exclusively reacts with carboxy moiety to produce activated intermediate *O*-acylisourea **3** rather than reacting with the amine (Scheme 2.4). The by-product dicyclohexylurea **4** is insoluble in most solvents and is easily removed by filtration. But occasionally, this insolubility could be problematic in polypeptide synthesis.[8] "Over activation" seems to be the only shortcoming of DCC method. The reactivity of *O*-acylisourea **3** is still too high, and some side reactions can compete. In addition, racemization tends to occur (Scheme 2.5).[8,9,10]

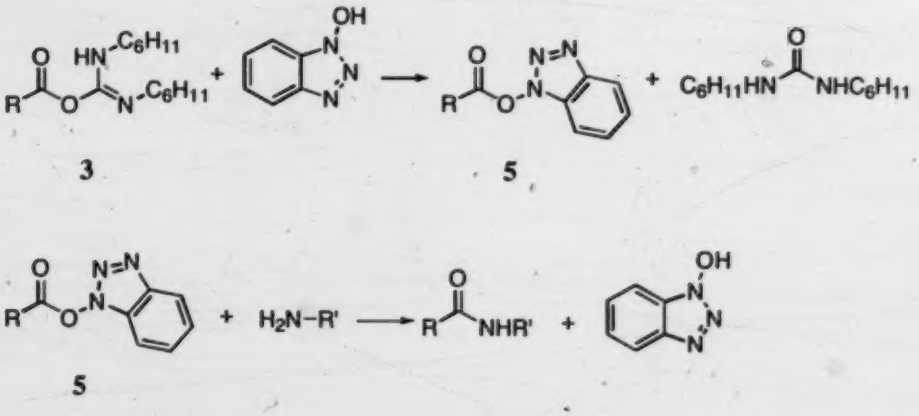


Scheme 2.4 Peptide bond formation using the coupling reagent DCC



Scheme 2.5 Racemization of peptides through oxazolones

Additives are used to decrease the reactivity of intermediate **3** without affecting the coupling process. The most common additive is HOBt which reacts with **3** to generate HOBt ester **5** (Scheme 2.6). An amine then attacks HOBt ester **5** to form the new peptide amide bond.



Scheme 2.6 Peptide bond formation using DCC/HOBt

Many types of coupling reagents and additives have been designed since DCC was introduced. A recent review by Han outlined these reagents and their applications.[8] Coupling reagents now include phosphoniums, uroniums, immoniums, carbodiimides,

imidazoliums, organophosphorous reagents, acid halogenating reagents and others. Some common reagents are depicted in Figure 2.1. For example, Schreiber and co-workers utilized BOP for the cyclization of a tetrapeptide to form a 12-membered ring tetrapeptide, a precursor of trapoxin B.[11] In addition the course of the synthesis of HUN-7293, a hindered tertiary amide bond, was generated using HOAT[12]. Generally, these coupling reagents are designed to reduce competitive formation of by-products, minimize racemization, or overcome steric effects.

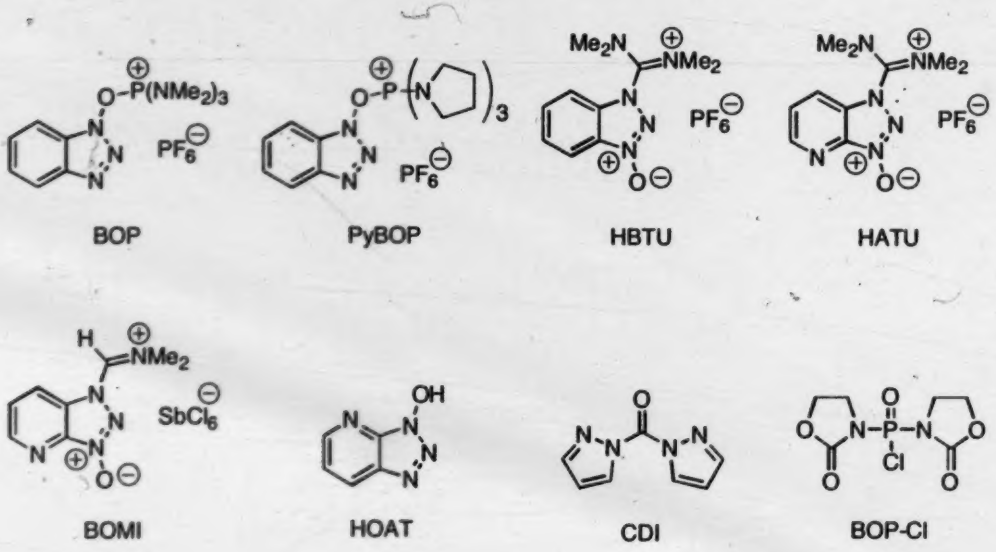
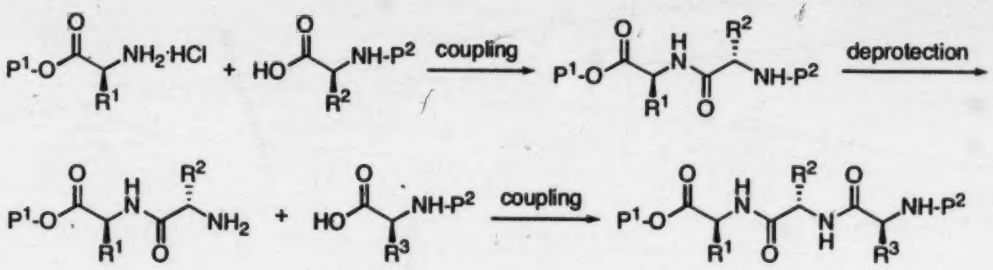


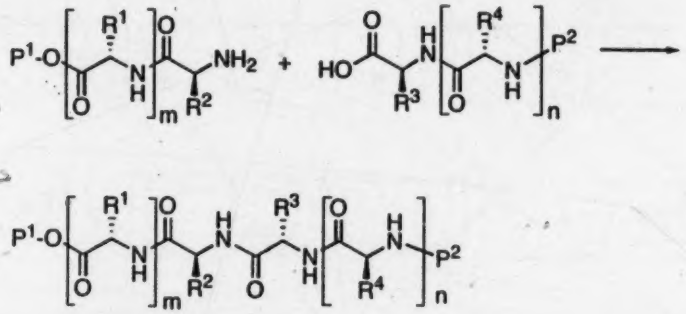
Figure 2.1 Coupling reagents for peptide amide bond formation

2.1.2 Strategies for forming long peptides

Peptide bonds can be elongated either in a stepwise fashion (Scheme 2.7) or by segment coupling strategies (Scheme 2.8). The stepwise approach is suitable for short peptide synthesis whereas segment coupling is better for long peptide synthesis. By sequentially condensing seven fragments, Hoffman and co-workers assembled an 81-unit peptide residue, which corresponds to positions 24-104 of the peptide chain of the enzyme ribonuclease T_1. [13]



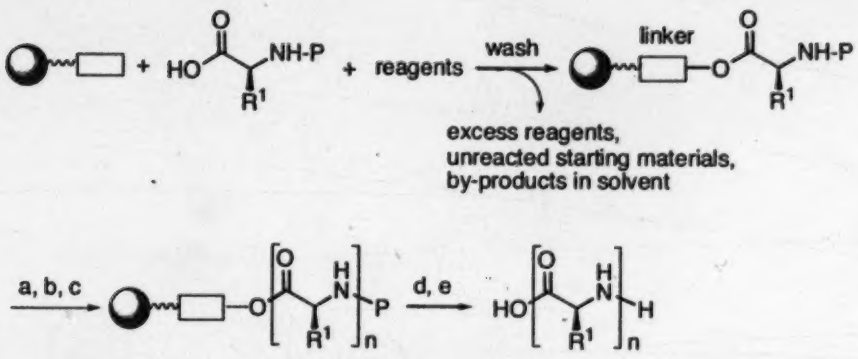
Scheme 2.7 Peptide bond formation by stepwise strategy



Scheme 2.8 Peptide bond formation by segment coupling strategy

2.1.3 Synthesis of peptides on solid-phase

Since the first report of peptide synthesis on solid phase by Merrifield in 1960s,[1] this technique has been extensively studied and applied in the synthesis of peptides and other compounds.[14] Through a linker, compounds are constructed onto a solid support (generally polystyrene). After a reaction, excess starting materials, reagents and by-products in solvent are simply washed away. The general procedure for the stepwise synthesis of linear peptides is shown in Scheme 2.9. Obviously, this makes purification easy and fast. Combined with the analytic techniques of the solid phase chemistry, peptide synthesizers (robots) are commonly used for quickly assembling thousands of compounds for drug discovery. Most coupling reagents in solution phase are also available for solid phase synthesis.



Reaction conditions: (a) remove protecting group P. (b) coupling with *N*-protected amino acid. (c) repeat step a and b. (d) remove protecting group P. (e) cleavage from the resin.
Note: R^1 could be different groups depending on amino acids

Scheme 2.9 General procedure of peptide synthesis on solid support

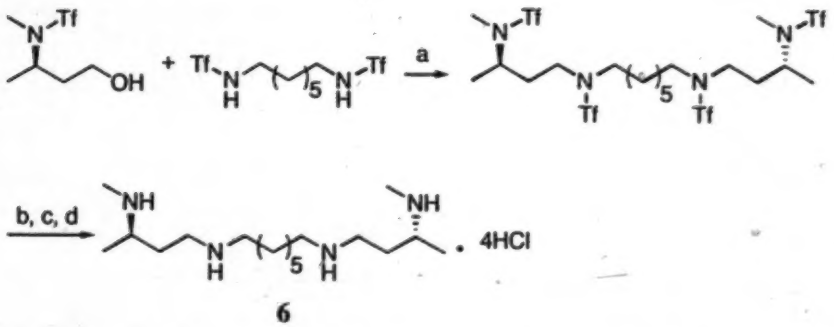
Both deprotection and coupling cycles are involved for each peptide bond extension, so monitoring each step is crucial before proceeding further. The Kaiser color test is the most widely used qualitative test for the presence or absence of free amino group.[15] A small amount of solid support (resin beads) is subjected to the Kaiser ninhydrin reagents. Free amino groups give a positive test (blue resin beads). If incomplete coupling occurs, pink resin beads are observed which indicate the presence of minimal amount of unreacted amino groups. Modern instrumentation such as HPLC-MS gives a more precise and quantitative monitoring of the peptide formation, albeit a sample of produt must be cleaved from the resin.

Compared with solution phase methods, peptide synthesis on solid phase has several advantages: 1) no separation and purification is needed before cleavage; 2) large excess of reagents can be used to force the reaction to completion. 3) assembly of compounds is fast; 4) the operation can be performed on peptide synthesizers. However, some limitations cannot be neglected, such as the possible accumulation of impurities. If in some cases by-products form in each step, extensive impurities will be observed at the final stage. The second limitation deals with aggregation that causes incomplete coupling, especially for long peptides, because of the secondary structures that some peptides

develop. A third disadvantage is large excess of reagents that must be used to force reactions to completion.

2.1.4 Synthesis of polyamines

Like the synthesis of peptides, mentioned before, polyamines can be synthesized in solution phase or on solid phase, and this topic was recently reviewed.[16, 17] With the help of amine protection and deprotection strategies, most common synthetic approaches in solution phase include alkylation of amines, Michael addition, Mitsunobu reaction and reduction of azides and Schiff's bases, and reduction of compounds that contain amide, nitrile and nitro groups. For example, Edwards reported the preparation of polyamine **6** via a Mitsunobu reaction (Scheme 2.10).[18]

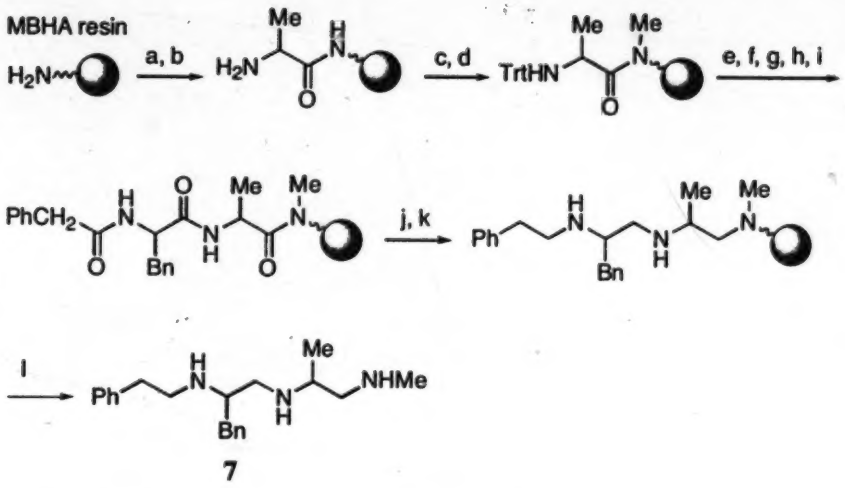


6

Reaction conditions: (a) PPh_3, DEAD, THF. (b) Na, NH_3, THF, *t*-BuOH. (c) $(t\text{-BuOCO})_2O$ (d) HCl, MeOH

Scheme 2.10 Synthesis of polyamine **6** by Edwards and co-workers[18]

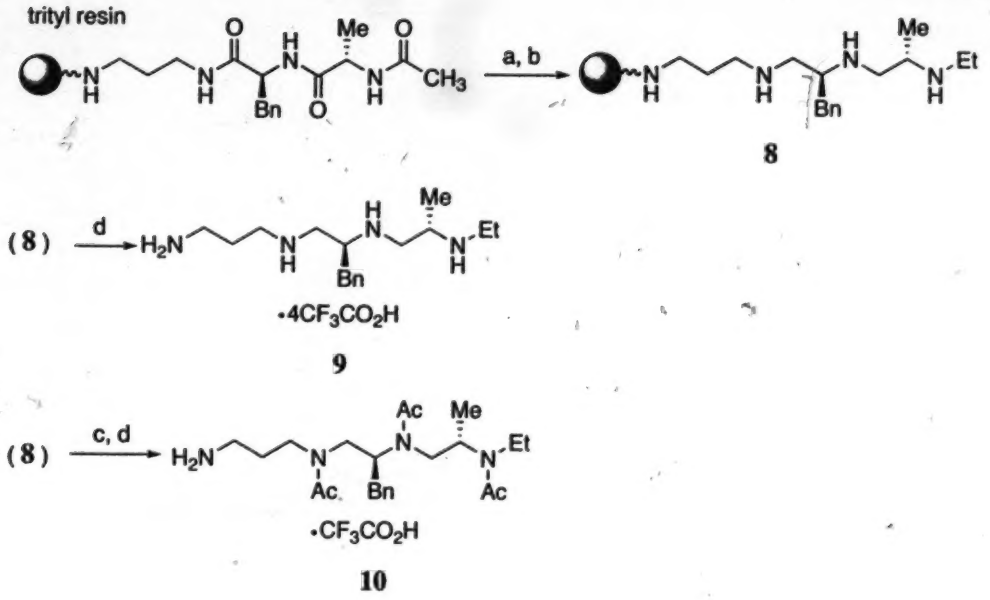
Solid phase synthetic approaches to polyamines by reduction of polyamides were reported by Houghten and co-workers[19] (Scheme 2.11) and by our group.[20, 21] Instead of using piperidine for work up, which needs long reaction times (16 h) and heating at 65 °C, our group developed a mild oxidative cleavage of borane-amine adducts by using I_2/*i*-Pr_2EtN/ AcOH (Scheme 2.12).



7

Reaction conditions: (a) Boc-Ala-OH, DIC, HOBt, DMF; (b) 55% TFA/DCM; (c) TrtCl, DIPEA, DCM; (d) MeI, *t*-BuOLi, DMSO; (e) 2% TFA/DCM; (f) DIPEA/DCM; (g) Fmoc-L-Phe-OH, DIC, HOBt; (h) 20% piperidine/DMF. (i) $PhCH_2CO_2H$, DIC, DMF. (j) BH_3/THF, 65°C (k) piperidine 65 °C. (l) HF/anisole

Scheme 2.11 Synthesis of polyamine **7** by Houghten and co-workers[19]

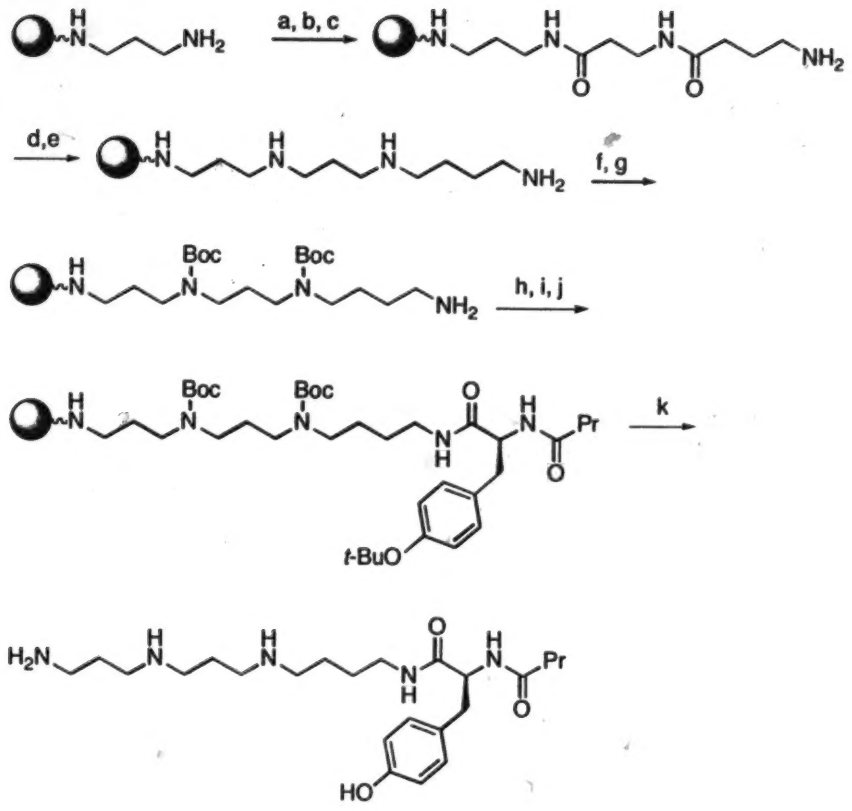


Reaction conditions: (a) 1M BH_3/THF, 65 °C, 24 h. (b) Iodine, THF/DIPEA/AcOH, rt, 4 h. (c) Ac_2O, Et_3N, DMF. (d) 5%TFA/DCM.

Scheme 2.12 Synthesis of polyamine **9** and acetylated polyamine **10** by Hall and co-workers[20, 21]

The groups of Hall, Jaroszewski and Bycroft have applied different strategies to generate polyamine chains on solid support for the synthesis of acyl polyamine philantotoxin-433 (PhTX-433) (**11**), a neuroactive constituent of the venom of *philanthus trangulum* digger wasp. [22, 23, 26]

Hall and co-workers have utilized the protocol in Scheme 2.12 to synthesize natural polyamines toxins HO-416b and PhTX-433 (**11**) (Scheme 2. 13).[22]



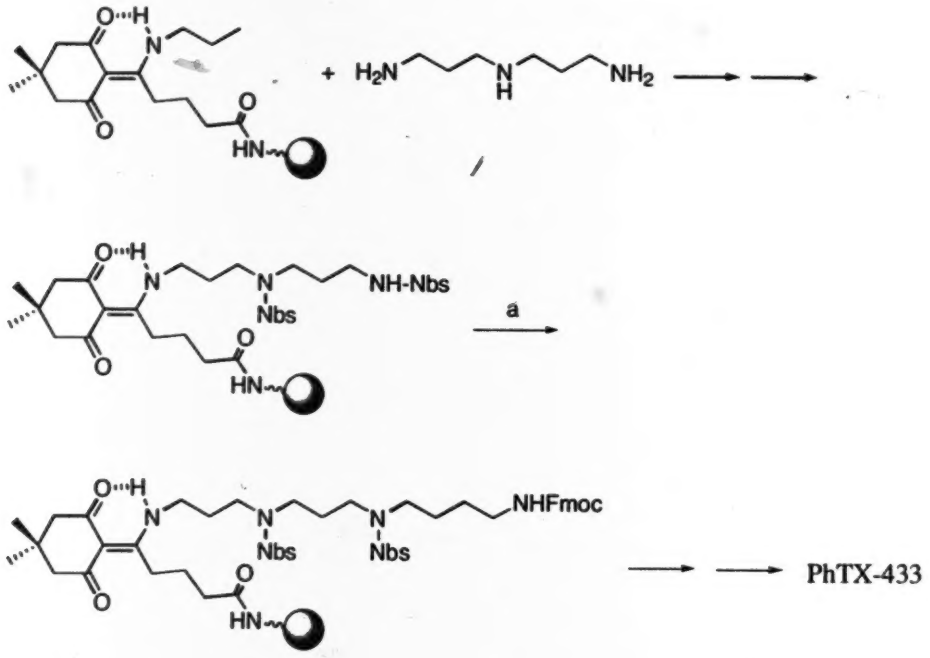
11

Reaction conditions: (a) $HO_2C(CH_2)_2NHFmoc$, HBTU, HOBt, DIPEA, DMF. (b) 20% piperidine in DMF. (c) repeat step a with $HO_2C(CH_2)_3NHFmoc$. (d) 1 M BH_3/THF, 65 °C, 48 h. (e) Iodine, THF/DIPEA/AcOH, rt, 4 h. (f) Dde-OH, DMF, rt, 2 h. (g) Boc_2O, DIPEA. (g) 2% NH_2NH_2/DMF. (h) HO-Tyr(*t*-Bu)-Fmoc, HBTU, HOBt, DIPEA. (i) 20% piperidine in DMF. (j) $CH_3CH_2CH_2CO_2H$, HBTU, HOBt, DIPEA. (K) TFA/H_2O/*i*-Pr_3SiH (95:2.5:2.5)

Scheme 2.13 Synthesis of PhTX-433 (**11**) by Hall and co-workers[22]

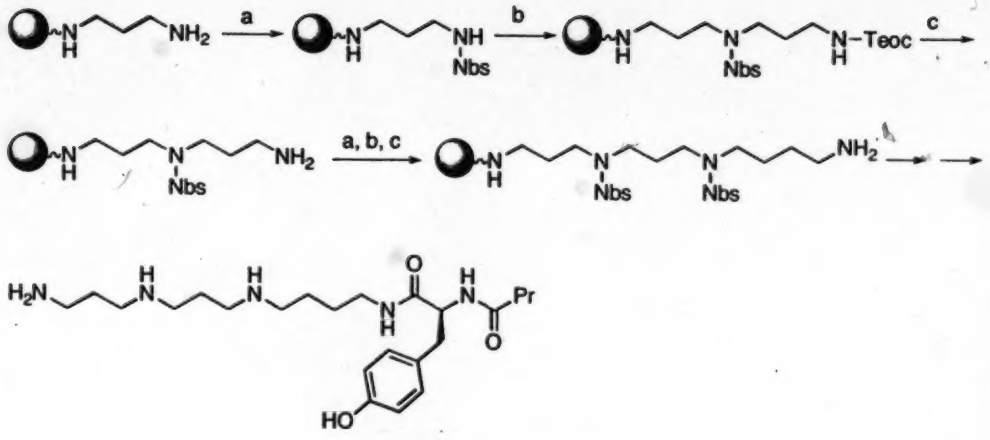
To extend a polyamine chain under Mitsunobu reaction conditions, Bycroft and co-workers used nitrobenzenesulfonamide strategies,[23] which was developed by the Fukuyama group.[24, 25] In Bycroft's protocol, an Fmoc amino alcohol was employed as a substrate (Scheme 2.14). A similar strategy was applied by Jaroszewski and co-

workers.[26] In their protocol, *N*-Teoc-protected 3-aminopropanol was used as a substrate to perform Mitsunobu reactions twice (Scheme 2.15).



Reaction conditions: (a) $FmocNH(CH_2)_4OH$ (10 equiv.), Ph_3P (5 equiv.), DEAD (5 equiv.), THF, 20 h.

Scheme 2.14 Synthesis of PhTX-433 (**11**) by Bycroft and co-workers[23]



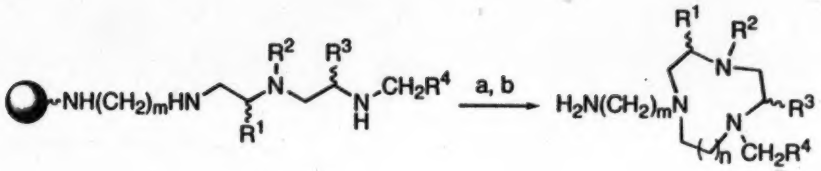
PhTX-433 **11**

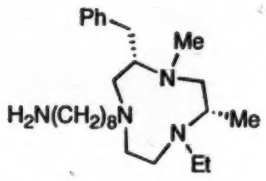
Reaction conditions: (a) 2-nitrobenzenesulfonyl chloride (4 equiv.), DIPEA (6 equiv.), THF, DCM, N_2, rt, 3 h. (b) 1,1'-(azodicarbonyl) dipiperidine (5 equiv.), *n*-Bu_3P (5 equiv.), *N*-Teoc-protected 3-aminopropanol (5 equiv.), THF/DCM, N_2, rt, 3 h. (c) TBAF (5 equiv.), THF, 50 °C, 3 h.

Scheme 2.15 Synthesis of PhTX-433 (**11**) by Jaroszewski and co-workers[26]

The Goodman group also applied the Mitsunobu reaction for the synthesis of amine-bridged cyclic enkephalin analogues using *N*-(allyloxycarbonyl)ethanolamine as substrate. [27]

Hall and co-workers reported the synthesis of chiral triazacycloalkanes on solid support. [28] After the final cyclization step, the product was released from trityl resin (Scheme 2.16). One example of the compounds synthesized was **12**.





12

Reaction conditions: (a) $TfOCH_2(CH_2)_nOTf$, DIPEA, DCM, rt, (n =1 or 2). (b) 5% TFA/DCM

Scheme 2.16 Synthesis of triazacycloalkane **12** by Hall and co-workers[28]

Like peptide synthesis, similar disadvantages and advantages between solution phase and solid phase methods were observed.

2.2 Perspective and objective of the project

The goal of this project is to synthesize exo-peptides and analyze their physico-chemical properties and their conformational behavior.

As discussed in Section 1.3 (Chapter 1), peptides and peptoids with proper backbone or side chains can display secondary structures. In our laboratory, we are seeking new types of biopolymers possessing secondary structures and biological activities. One such type of compounds was the family of acetylated polyamines (exo-peptides) (Figure 2.2). In principle, because of their tertiary backbone amide structures, these molecules will exist as in a mixture of rotamers. It is risky to predict the existence of secondary structure without the use of advanced molecular modeling techniques. Nonetheless, from the simple analysis shown in Figure 2.3 where each unit is dissected into three dihedral angles (α, β, γ), it seems that the relatively large side chains could induce a preference for one or two favorable rotamers of angle α that minimize gauche

interactions. If this analysis is correct, in a mixture of four rotamers (A, B, C, D) (Figure 2.3), rotamer A should help favor helical structures. For angles β and γ, preference for rotamers C and D with the main chain expected to be orthogonal to the amide plane, irrespective of the stereoregularity of these tertiary amides, should help favor backbone turns. Exo-peptides that are made of tertiary amides should resist the action of proteolytic enzymes *in vivo*. Moreover, because they lack a hydrogen donor functionality, they should be relatively hydrophobic and, thus, be highly membrane permeable.

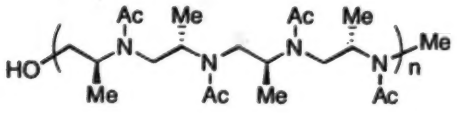


Figure 2.2 Structures of exo-peptides

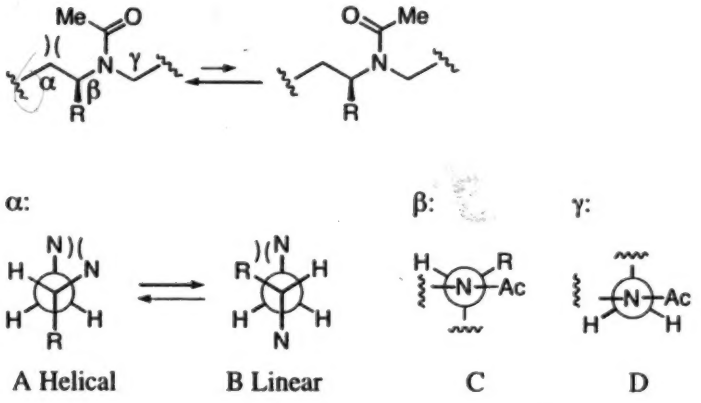
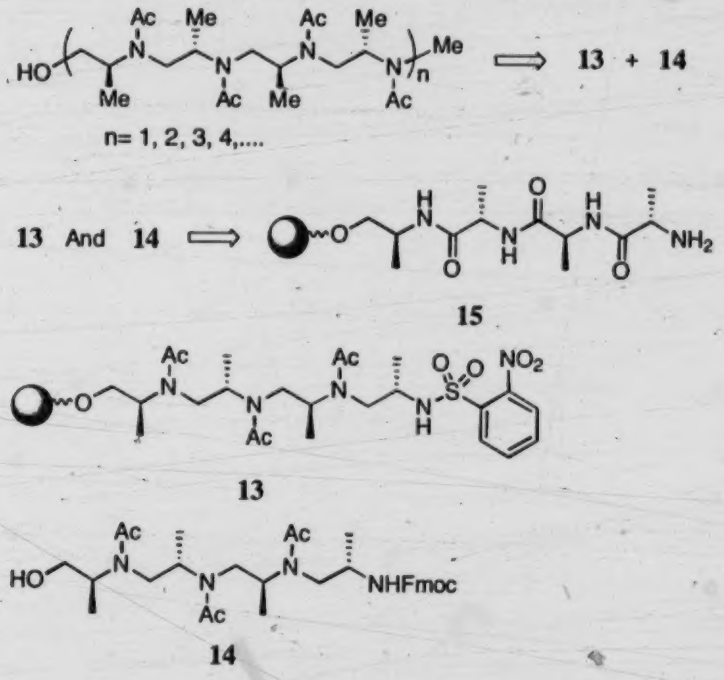


Figure 2.3 Conformational analysis of exo-peptides

Two possible approaches for the synthesis of exo-peptides were identified: 1) acetylation of polyamines; 2) coupling of a short amine and an amino alcohol under Mitsunobu reaction conditions. For approach 1, reduction of long peptides could afford polyamines. There are two potential challenges with this approach, which include construction and exhaustive reduction of long homopeptides. Although many peptide synthesis methods have been developed (Section 2.1), very few syntheses of

homopeptides, especially long ones, have been reported. The low solubility of long homopeptides for solution phase methods and aggregation in the solid-phase are the main reasons.[29, 30] At first sight, approach 2 seems feasible. The Mitsunobu reaction was successfully applied for the synthesis of polyamines or polyamine conjugates (Section 2.1.4).

The Mitsunobu reaction and our solid-phase polyamine synthesis protocol[20, 21] lent promise for this project. It was known that long peptides with more than four amide carbonyl groups are incompletely reduced by BH_3-THF on the solid phase. So, a feasible plan would involve the construction of a shorter resin bound tetraamine first, followed by chain extension under Mitsunobu reaction conditions. The exo-peptide could be generated by coupling **13** and **14** under Mitsunobu reaction conditions on the solid-phase. Both **13** and **14** could come from the same resin bound peptide **15** through a few synthetic conversions (Scheme 2.17).



Scheme 2.17 Retrosynthetic approach to exo-peptides

2.3 The synthesis of exo-peptides

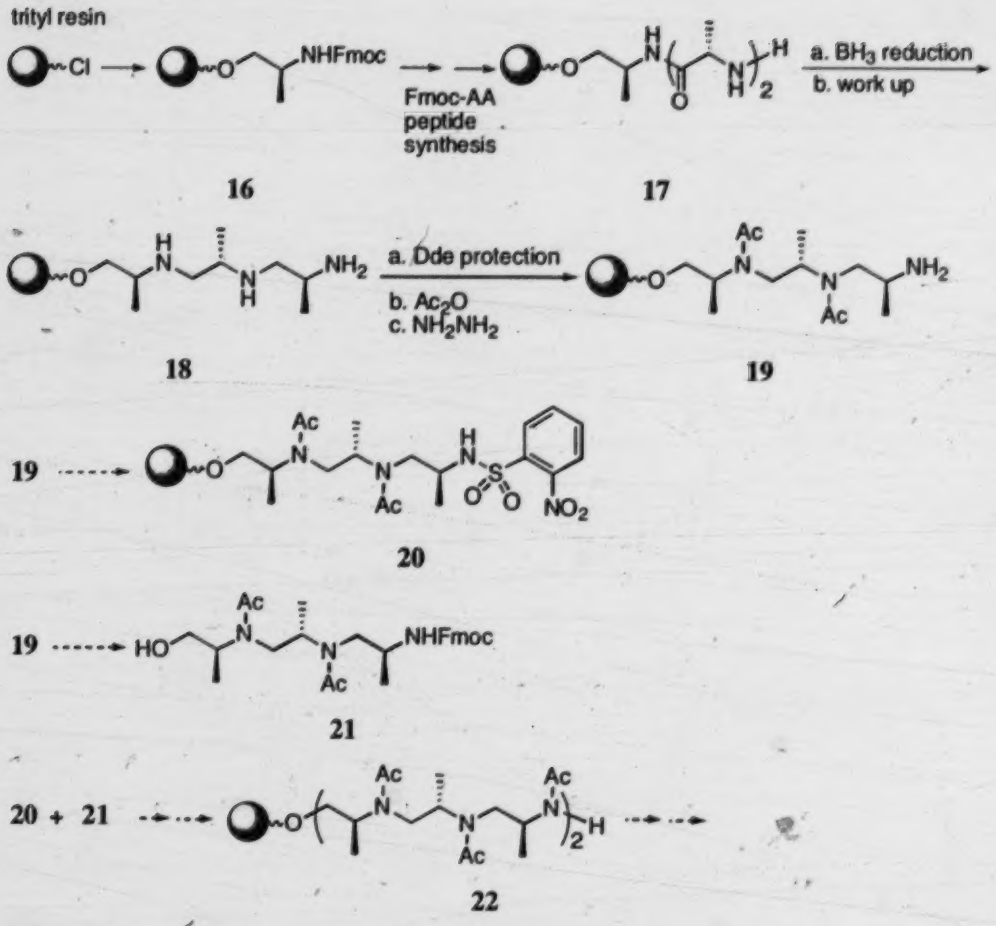
As discussed in Section 2.1, peptides are synthesized either in solution phase or on solid phase. The advantages of solid phase synthesis prompted us to synthesize exo-peptides on solid support as a first trial.

2.3.1 The synthesis of exo-peptides through solid phase chemistry

Hall and co-workers[21] have prepared short acetylated tetraamines through a solid phase approach (Scheme 2.12). Theoretically, utilizing the same processes and building long peptides on solid support, followed by reduction with diborane and acetylation, exo-peptides should be generated. In practice, there were some challenges. First, there was incomplete reduction of amide bonds on the resin. If there were more than four amide carbonyl groups, incomplete reduction of amide bonds by diborane was inevitable,[33] even when a large excess of reducing reagent and prolonged reaction time was applied. Another potential problem could be aggregation of long peptides on solid support. This will be discussed at a later stage. As mentioned in Section 2.2, Mitsunobu coupling of amines and alcohols seemed more feasible.

2.3.1.1 Mitsunobu strategy using C-O bonds as resin anchors

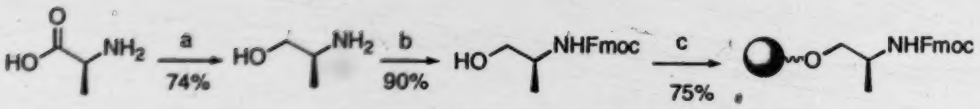
The outline of this approach is shown in Scheme 2.18. A resin bound acetylated triamine alcohol **19** could be constructed from acetylation of resin **18**, which could be prepared from trityl chloride resin. Both substrates **20** and **21** for Mitsunobu reaction could be generated from intermediate **19**. If this strategy worked, it was hoped to extend the acetylated polyamine by three units via each coupling process.



Scheme 2.18 Synthetic approach using a Mitsunobu coupling strategy

2.3.1.1.1 Synthesis of resin-bound Fmoc-L-alaninol (16)

L-Alaninol was obtained as a colorless oil in 74% yield by reduction of L-alanine with $LiAlH_4$ in THF followed by distillation.[31] Fmoc-L-alaninol (**23**) was prepared by treatment of L-alaninol with Fmoc-OSu giving a 90% yield.[32] The ^{1}H-NMR spectra of L-alaninol and Fmoc-L-alaninol (**23**) were consistent with literature data.[31, 32] The resin-bound Fmoc-L-alaninol (**16**) was achieved by loading Fmoc-alaninol (**23**) to trityl resin using literature procedures.[33]



23 **16**

Reaction conditions: (a) $LiAlH_4$/THF, 0 °C then 75 °C, overnight. (b) Fmoc-OSu (1 equiv.) in THF, 10% aq. Na_2CO_3, 0 °C to rt, pH = 9. (c) trityl chloride resin (1 equiv.), **23** (4 equiv.), pyridine (4 equiv.), rt. 4 days.

Scheme 2.19 Synthesis of resin bound Fmoc-alaninol (**16**)

There are several reasons to select the trityl chloride polystyrene resin (trityl chloride resin) as a solid support. First, compounds immobilized on this resin can be cleaved under mild conditions (5% TFA/DCM). Second, the linker is stable under basic conditions during coupling and deprotecting steps. For solid phase synthesis, loading and cleavage are crucial. At the beginning of this project, it was necessary to ensure that these two steps worked well. Unlike the amine nitrogen, the alcohol oxygen is not nucleophilic enough, so loading Fmoc-alaninol onto trityl resin was slow. Wenschuh and co-workers[33] evaluated loading efficiency vs reaction time. They immobilized Fmoc-phenylalaninol and Fmoc-valinol onto 2-chlorotrityl chloride resin and found that it took one day for 70% and two days for 80% loading efficiency. In our case, it took four days for 75% loading efficiency at room temperature. At 75 °C, the same loading efficiency was reached in 4 h (Table 2.1). Room temperature conditions were preferred to avoid destruction of the resin. Loading efficiency was calculated by loading level, which was measured by UV analysis or elemental analysis. A large amount of Fmoc-alaninol was needed to get high loading efficiency. However excess Fmoc-alaninol was recoverable by extraction of the reaction solution.

Table 2.1 Loading of Fmoc-alaninol to trityl chloride resin

entry	resin[i] (mmol/g)	condition	molar ratio[ii]	loading efficiency %
1	0.95	75°C, 2h	1 : 2 : 4	49%
2	1.38	75°C, 22h	1 : 2 : 4	73%
3	2.05	rt, 4 days	1 : 4 : 4	76%
4	2.05	75°C, 22h	1 : 2 : 4	75%

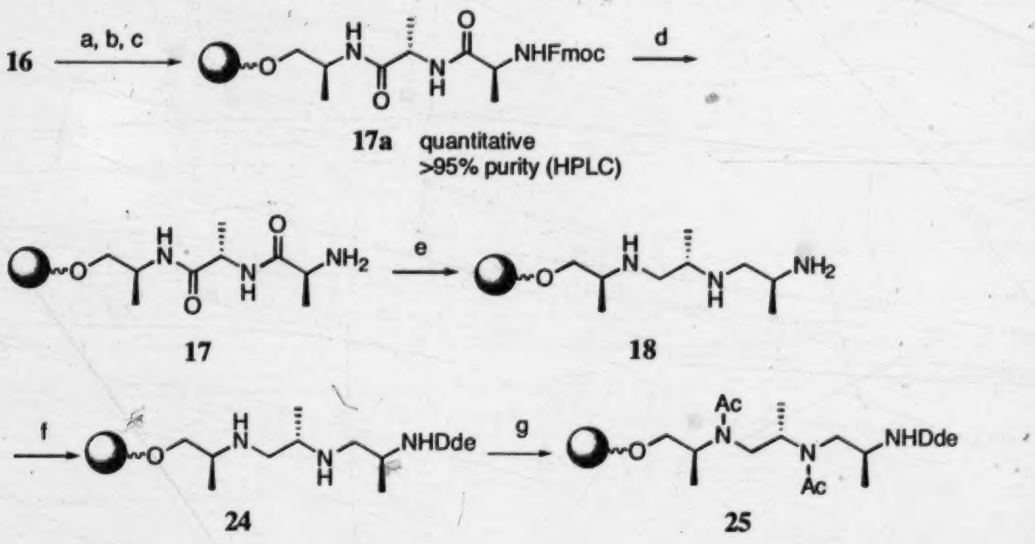
Note: i) resin: purchased from Novabiochem
ii) molar ratio = resin : Fmoc-alaninol : pyridine

Cleavage of alcohols from the trityl resin can be achieved with PTSA in THF/MeOH,[34] HCl in dioxane, [35] TFA in DCM, [36] or HCO_2H/THF.[37] Comparing the HCl and TFA methods, TFA gave the desired compound with higher purity. There were two main peaks observed by LC-MS. One peak represented Fmoc-L-alaninol (*m/z* $(M+Na)^+$ = 320.2) and the other represented Fmoc-L-alaninol TFA ester (*m/z* $(M+Na)^+$ = 416.1). The ratio of the TFA ester depends on the cleavage time and concentration of TFA. There was a necessity to find a way to convert TFA esters to the free alcohols because TFA esters can not be used as Mitsunobu reaction substrates in this strategy. Gratifyingly, TFA esters can be cleaved by treatment with sodium carbonate or dimethylethylamine. Treatment with dimethylethylamine (5% in MeOH) at room temperature for 30 minutes effectively converted the TFA ester to the alcohol. Interestingly, leaving the LC-MS sample (in MeOH) for 3 days, followed by re-injection showed a lower TFA ester peak and complete disappearance of the peak occurred after 5 days. This was due to transesterification between methanol and Fmoc-L-alaninol. At this point, the loading and cleavage steps were of an acceptable standard.

2.3.1.1.2 Synthesis of resin bound peptide 18 and 24

Standard Fmoc-amino acid coupling (Fmoc AA) generated resin bound Fmoc-Ala-Ala-Alaninol (Scheme 2.20). Treatment of resin **16** with 20% piperidine in DMF for 30 minutes removed the Fmoc group. Coupling reagents HBTU/HOBt promoted peptide

bond formation. After coupling twice, both negative Kaiser tests and LC-MS results showed complete coupling. (Fmoc-Ala-Ala-Alaninol: m/z = 440.2). From the ^{1}H NMR spectra in $CDCl_3$, it was found that the three methyl groups overlapped. After the Fmoc group was removed to generate resin bound peptide **17**, no overlap of the methyl groups was observed in the NMR spectra.



Reaction conditions: (a) 20% piperidine/DMF (b) Fmoc-L-alanine, HBTU, HOBt, DIPEA. (c) repeat (a) and (b). (d) 20% piperidine/DMF, rt, 30 min. (e) (i) 1M BH_3-THF (20 equiv.), THF, 65 °C, 18 h. (ii) piperidine, 65 °C, 16 h. (f) resin **18** (1 equiv.), Dde-OH (1.1 equiv.), Et_3N (4 equiv.), DMF, rt, 3 h. (g) resin **24** (1.0 g), Ac_2O (1 mL), Et_3N (0.4 mL), DMF, rt, 3 h.

Scheme 2.20 Synthesis of resin bound triamines **18**, **24** and **25**

Resin-bound triamine **18** was obtained by standard BH_3-THF reduction of resin **17**.[21, 28] The Kaiser test was not reliable in this case as a purity test since partial reduction products, containing amide carbonyl groups, can also give a positive test. LC-MS was also not reliable. MS spectra did show a signal for the triamine, but in the UV spectra, no corresponding signal was found, since amines are not UV sensitive. A UV sensitive group can be attached to the triamine in order to check the reduction product. The Dde

group was used to protect the primary amine for two reasons. Firstly, Dde-OH and its enamine derivatives are UV sensitive and stable under TFA cleavage conditions,[38, 39] allowing for indirect determination of purity of the triamine. Secondly, the next step of this approach was Dde protection of the primary amine. LC-MS showed a high purity (95%) for the Dde protected triamine, but results of the elemental analysis indicated that more than 80% of the compound was cleaved in the reduction step. Other reduction conditions were also investigated (Table 2.2).

Table 2.2 BH_3-THF reduction of resin bound peptide **17**

	Reagent (equivalence to resin)	Time	Resin Before reduction		Resin After reduction		Purity[i]	Yield[ii]
			mg	N%	mg	N%		
1	BH_3-THF 20	18 h	124	5.78	61	0.67	>95%	5.7%
2	BH_3-THF 20	18 h	425	5.78	268	0.79	>95%	18%[iii]
3	BH_3-THF 40 $B(OH)_3$ 12 $B(OMe)_3$ 12	18 h	127	5.78	67	0.44	>95%	4.0%
4	BH_3-THF 10 $HC(OMe)_3$ 20	4 h	57	5.78	53	5.16	50%	
5	BH_3-THF 23 $HC(OMe)_3$ 60	30 h	271	5.78	179	4.66	65%	50%
6	BH_3-Et_3N 20	28 h	57	5.78	52.7	5.79	no reaction	
7	9-BBN 20	18 h	78	5.79	78	----	no reaction	

Note: i: Purity was determined by LC-MS after Dde protection

ii: Calculation based on amount of nitrogen left after reduction

iii: Corresponding to 21.8 mg crude compound from 212 mg resin

Alternative reduction conditions by Houghten and co-workers[40] (BH_3-THF/$B(OH)_3$ / $B(OMe)_3$) provided a similar result. This could be due to the strong coordination ability of boron with the ether oxygen or trace water or acid in the system, which accelerated the cleavage. Therefore, a hindered reducing reagent, 9-BBN and basic conditions, BH_3-Et_3N were tried, but no reduction occurred. When BH_3-THF/ $HC(OMe)_3$ was used, 5% of

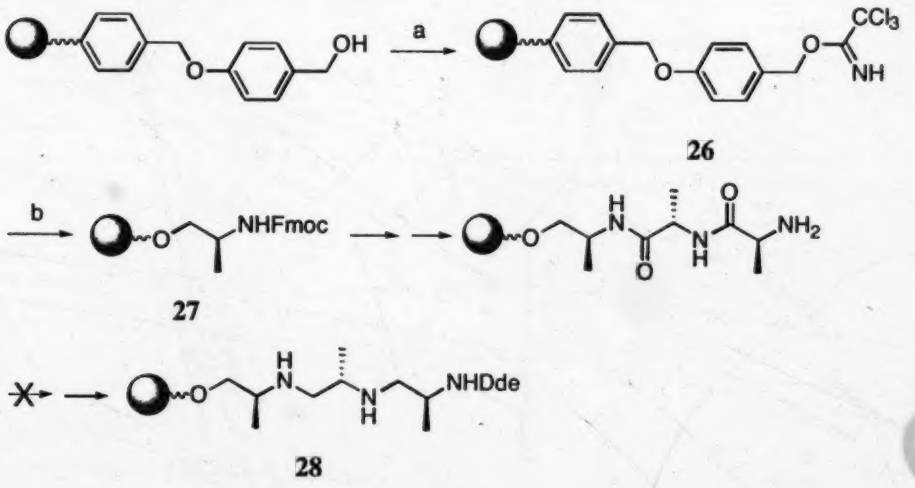
the compound was cleaved from the resin, but the purity was only 50% because of incomplete reduction. Increasing the reaction time sacrificed the purity (Table 2.2). In sum, it can be seen that the alkoxy trityl anchor was not resistant to BH_3-THF.

Acetylation conditions of polyamines were also investigated. The acetylation conditions of Scheme 2.12 provided resin **25** in 95% yield. Heating and addition of DMAP did not improve the yield.

2.3.1.1.3 Study of C-O ether bonds under reduction conditions on Wang resin

Compared with other resins, trityl resin is very acid labile because the resulting trityl cation is stabilized by three phenyl groups, which causes the release of compounds from trityl resin easily under mild conditions. It was also hoped that other resins on which the C-O bond was resistant to the reduction conditions, could be found.

Based on known procedures,[41] Fmoc-L-alaninol was successfully immobilized onto Wang resin to give resin **27**. The same sequence in Scheme 2.20 was followed to prepare Wang Resin-bound polyamine **28** (Scheme 2.21).



Reaction conditions: (a) Wang resin (1 equiv.), Cl_3CCN (18 equiv.), DBU (1 equiv.), DCM, 0 °C, 40 min. (b) Fmoc-L-alaninol (2 equiv.), $BF_3{\cdot}Et_2O$ (catalytic), THF. loading efficiency: 74%

Scheme 2.21 Construction of Dde protected triamine on Wang resin

Similar disappointing results were observed in the reduction step (Table 2.3). A possible reason as illustrated in Figure 2.4 may be that the two ether oxygen atoms and two phenyl groups in Wang resin promoted release of the compound from the resin. Replacement of one ether oxygen by a nitrogen atom could avoid this problem. This idea led to evaluation of a new linker.

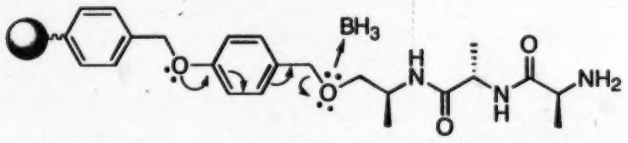
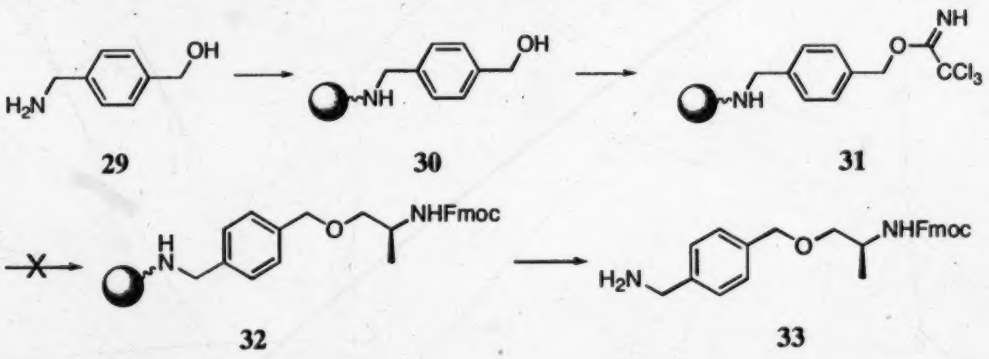


Figure 2.4 Hypothetical mechanism of release of compounds from Wang resin

2.3.1.1.4 New linker (4-hydroxymethylphenyl methylamine)

The resin **30** was designed because of three reasons: 1) it has a nitrogen anchor to trityl resin that should be stable to BH_3 reduction; 2) hydroxymethylene can be used for ether bond formation through the trichloride acetonitrile method used with Wang resin; 3) benzylic linker removed after TFA cleavage by hydrogenolysis.



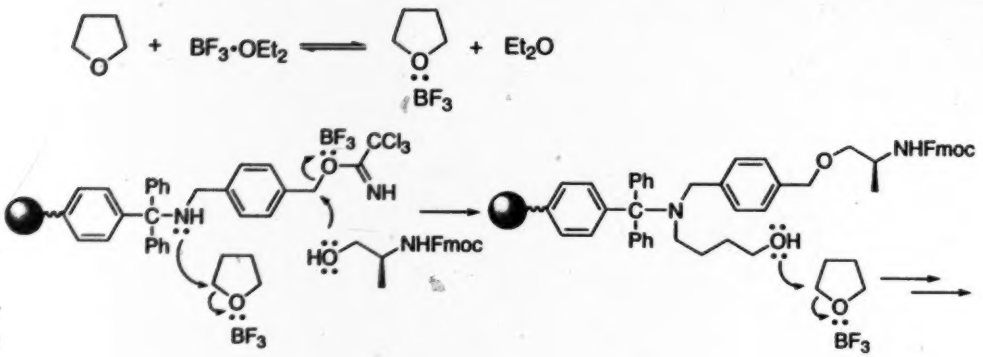
Scheme 2.22 4-Hydroxylmethylphenyl methylamine as a linker

Resin **30** was prepared by treatment of trityl chloride resin with 4-hydroxyl-methylphenyl methylamine (**29**), which in turn was produced by reducing 4-aminomethyl

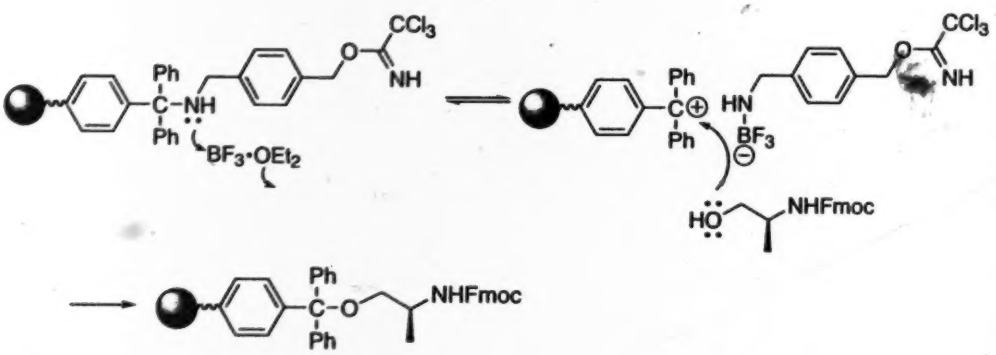
benzoic acid with $LiAlH_4$ (Scheme 2.22).[42] Successful loading was proven by checking the residue, which was cleaved from resin **30**. A peak (*m/z* =180.0) in LC-MS was observed, which corresponded to the linker **29**. The same procedure as in Scheme 2.21 was applied to load Fmoc-alaninol. Many strange peaks with equal intervals in UV and MS spectra (*m/z* = 417.1+ n*72) were observed in LC-MS analysis, although the largest peak (*m/z* = 417.1) was the desired compound **33**. In an attempt to discover the reason for the strange peaks and to further improve this sequence, the residue was cleaved from resin **32** using TFA and analyzed by ^{1}H-NMR and HR-MS. In the HR-MS spectra, peaks matched the molecular formula pattern: $C_{26}H_{28}N_2O_3$ + $(C_4H_8O)_n$ which is more likely the desired compound **33** plus polymerized 1,4-butylene diol. In the ^{1}H-NMR spectra, two equal large broad peaks (1.6 and 3.4 ppm) matched the $(CH_2CH_2CH_2CH_2O)_n$ pattern. Due to the tentative conclusion that THF might be involved through its possible ring opening occurring to form polymers, more stable solvents like DCM, DMF and DME were used for loading. Unfortunately, only trace amounts of desired compound **33** were obtained. Instead, Fmoc-alaninol was the major compound, as well as the free linker **29**.

To investigate this problem further, resin **32** was thoroughly washed and the solution was collected, no Fmoc-alaninol and linker **29** were found by LC-MS. It can, therefore, be seen that the Fmoc-alaninol and linker **29** did in fact come from resin **32**. It is therefore not possible that under the cleavage condition the ether bond was breaking to form Fmoc-alaninol and **29** from resin **32**, because when THF was used as a solvent, the ether bond is stable and the desired compound **33** was obtained as major product. One different phenomena observed, compared to using Wang resin, is that when $BF_3{\cdot}Et_2O$ was added to resin **31**, the color of the resin changed red or orange from slightly yellow depending on the concentration of $BF_3{\cdot}Et_2O$.

It is mostly likely that when THF was used as a solvent, there was an opening of the furan ring; when a stable solvent, such as DCM, was used as a solvent, the amino alcohol linker was released. The possible mechanism is proposed for the observation of oligomers of MW 72 (Scheme 2.23 and Scheme 2.24).



Scheme 2.23 Hypothetic mechanism of the loading step of resin **31** in THF



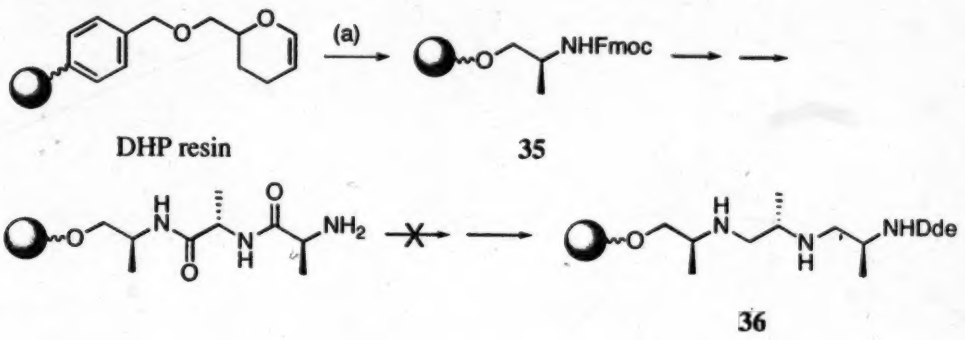
Scheme 2.24 Hypothetic mechanism of the loading step of resin **31** in DCM

If this mechanism holds, avoiding the use of $BF_3 \cdot Et_2O$ could prevent undesired compound formation. Thus, ether bond formation, via 2-pyridylthiocarbonate,[43] tosylate or alkyl iodo leaving groups,[44] was exploited, but no desired compound **33** was obtained.

2.3.1.1.5 Studies of the C-O ether bond as a linker on other resins under reduction conditions

A study to find a resin on which the C-O ether linkage bond is resistant to reduction conditions was then undertaken. Possible resins could be 3,4-dihydro-2H-pyran-2-yl-methoxymethyl polystyrene (DHP) resin and diphenyldiazomethane resin because these resins are less capable of forming a stable cation compared to trityl resin.

Fmoc-alaninol was loaded onto DHP resin following literature procedures (Scheme 2.25).[45, 46] The same sequence as in Scheme 2.20 was followed to prepare DHP resin-bound polyamine **36**.

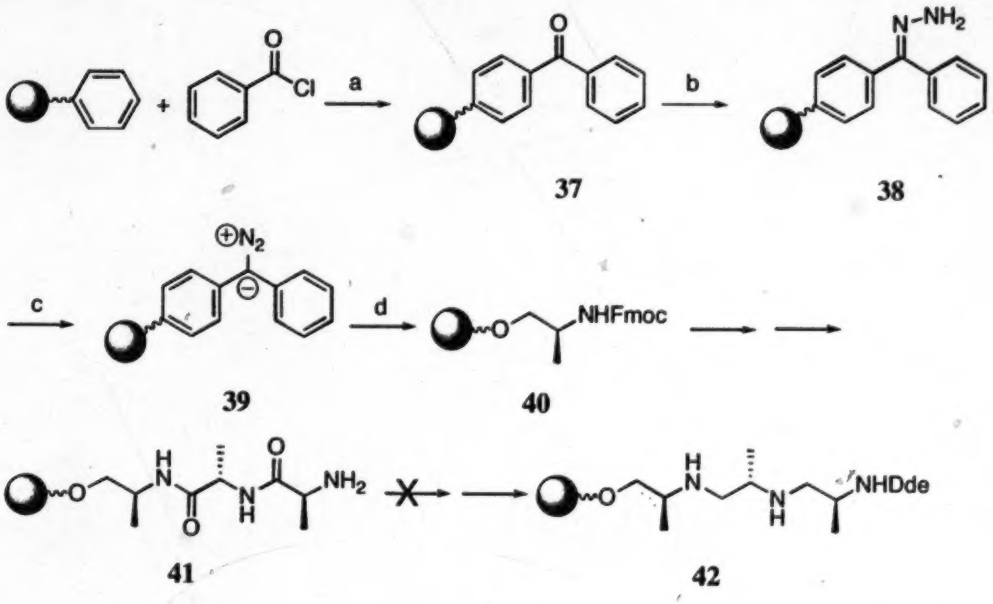


Reaction conditions: (a) PPTS (1.5 equiv.), Fmoc-L-alaninol (3 equiv.), DCE, N_2, 70 °C, overnight. Loading efficiency: 90%

Scheme 2.25 Attempted preparation of Dde protected triamine onto DHP resin

Diphenyldiazomethane resin **39** was not commercially available, so it had to be prepared from a polystyrene polymer. No detailed procedure was provided in the literature.[47] Benzoyl phenyl resin **37** was prepared according to early literature [48] by benzoylation of styrene divinylbenzene promoted by $AlCl_3$ (Scheme 2.26). A sharp, strong absorption peak (1659 cm^{-1}) in the IR spectra of resin **37** confirmed the presence of a carbonyl group. Conversion of resin **37** to hydrazone resin **38** was achieved by treatment of resin **37** with hydrazine hydrate.[49] The IR spectra showed NH_2 bond absorptions at 3411 and 3328 cm^{-1}. Hydrazone resin **38** was reacted with tetramethyl-guanidine and *m*-CPBA providing the deeply violet colored resin **39**.[47, 50] $BF_3 \cdot Et_2O$ mediated the loading of Fmoc-alaninol to resin **39**, generating resin bound Fmoc-alaninol **40**. Once the first loading was completed, the rest of the assembly was furnished according to the same procedure in Scheme 2.20.

Unfortunately, like tiryl resin, most compound was cleaved from the DHP resin and diphenyldiazomethane in the reduction step.



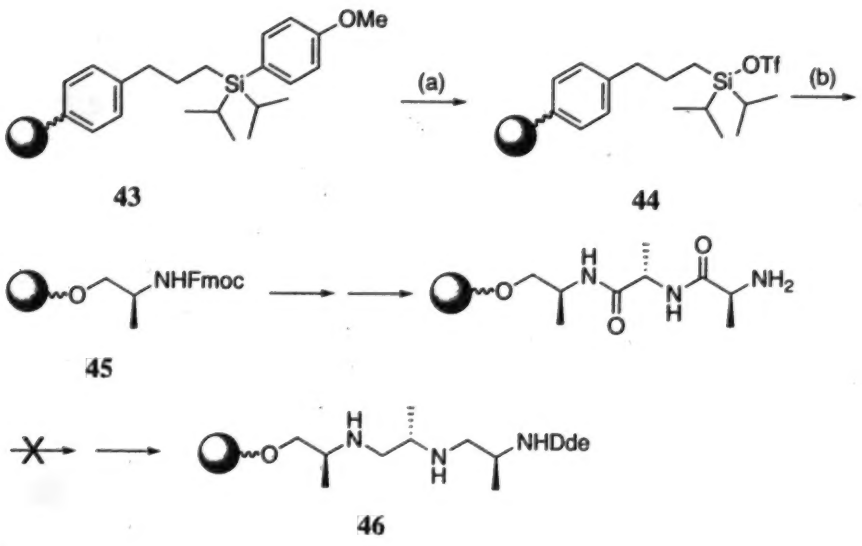
Reaction conditions: (a) polystyrene-divinylbenzene (1 equiv.), benzoyl chloride (2 equiv.), $AlCl_3$ (2 equiv.), N_2, -10 °C, 30 min. then, rt, 3.5 h. (b) $NH_2NH_2 \cdot H_2O$ (10 equiv.), *n*-BuOH, 120 °C, 16 h. (c) tetramethylguanidine (3.5 equiv.), *m*-CPBA (1.3 equiv.), I_2 (catalytic), DCM, N_2, -10 to 0 °C. (d) Fmoc-L-alaninol (2 equiv.), $BF_3 \cdot Et_2O$ (0.1 equiv), DCM, rt, 4 h.

Scheme 2.26 Attempted preparation of Dde protected triamine onto diphenyl-diazomethane resin

In some way, the concepts of development of the resin came from protecting group strategies. Loading and cleaving compounds in solid-phase chemistry is similar to protecting and deprotecting functional groups, for example, the DHP resin is analogous to protecting alcohol groups using THP in solution phase. Based on this, if one kind of alcohol OH protecting group survives during reduction in solution phase, the corresponding resin should have similar properties. Tanaka and coworkers reported that TMS protecting groups can survive the strong reducing agent $LiAlH_4$.[51] In 1998, Porco and co-workers reported that alcohols can be immobilized to a trialkylsilyl resin.[52] This resin was resistant to $LiBH_4$ and $NaBH(OAc)_3$ reduction conditions.[53, 54] This resin may also be tolerant to BH_3-THF. Therefore, Fmoc-alaninol was loaded onto silyl resin **73**

following a literature procedure (Scheme 2.27).[55, 56] Again, most of the compound was released from the resin during reduction with BH_3-THF. Reduction with $LiAlH_4$ resulted in cleavage of all of the material from the resin.

As a note, all coupling steps and transformation steps of the Wang resin, DHP resin, diphenyldiazomethane resin and silyl resin were monitored by Kaiser tests and LC-MS. Loading efficiency was measured either by UV or elemental analysis, and the purity of reduction compound was measured by HPLC-UV (Table 2.3).



Reactions: (a) silyl resin (1 equiv.), TfOH (6 equiv.), DCM, rt, N_2, 1.5 h. (b) 2,6-lutidine (6 equiv.), Fmoc-L-alaninol (2 equiv.), DCM, rt, N_2, 10 h.

Scheme 2.27 Attempted preparation of Dde protected triamine onto silyl resin

Table 2.3 Reduction of peptides on various resins

	resin	initial loading level mmol/g	loading efficiency %	yield[i] %	purity %
1	Wang resin	1.07	82	73	64
2	Wang resin	1.07	80	32	> 90
3	DHP resin	0.83	91	28	> 90
4	Diphenyldiazomethane resin	0.61[ii]	-	51	67
5	silyl resin	1.4	91	34	> 90

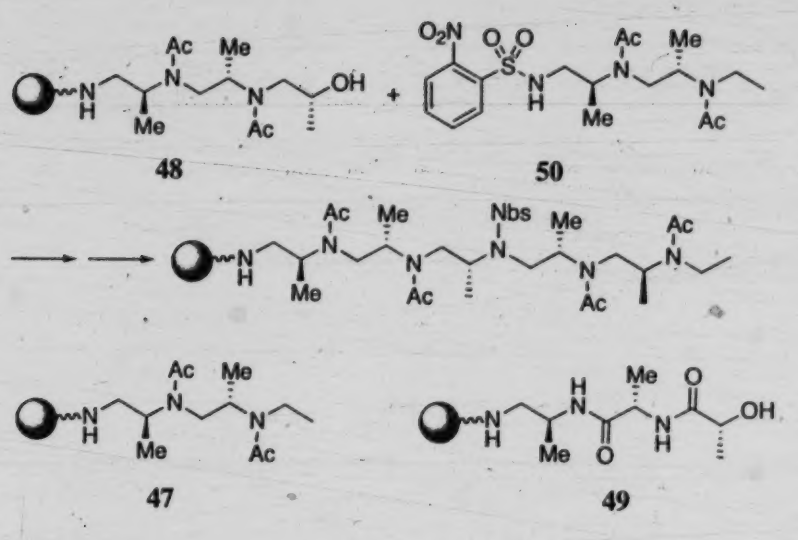
Note: i) yield was based on elemental analysis of nitrogen of resin

ii) loading level was calculated based on the elemental analysis of nitrogen of resin **41**

In the end, no linker containing a C-O ether attachment to the resin was found to be resistant to BH_3-THF reduction conditions. An alternative approach of the syntheses of Mitsunobu reaction substrates on solid support would be the use of C-N bonds as linkers, which are known to be stable under reduction conditions.

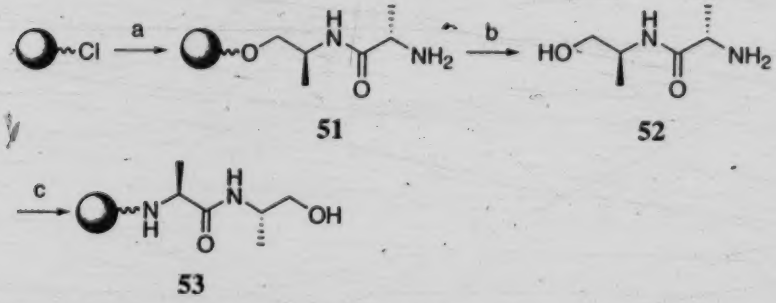
2.3.1.2 Mitsunobu strategy using C-N bonds as resin anchors

As shown in Section 2.1.4, a C-N linker for the attachment of a compound to trityl resin is stable and tolerant to reduction conditions, so it could be possible to run the Mitsunobu reaction using resin **48** and Nbs protected acetyl amine **50** which could, in turn, be generated from resins **47** and **49** respectively (Scheme 2.28). Resins **47** and **49** could be prepared from trityl chloride resin using a procedure similar to that of Scheme 2.12.



Scheme 2.28 C-N bonds as linkers for Mitsunobu strategy

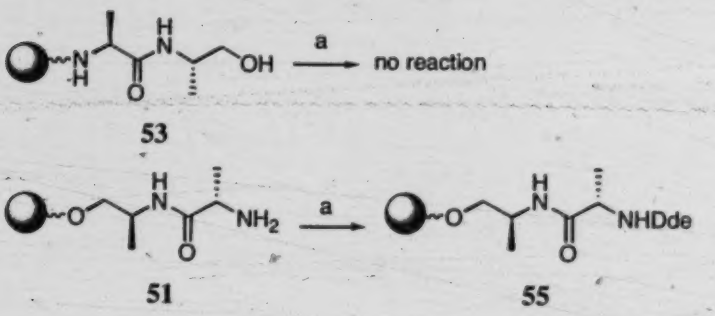
To evaluate this strategy, a model study was needed. A simple resin-bound compound **52** was chosen. **53** was prepared by re-attaching L-alanine-L-alaninol (**52**) to trityl resin by the amine nitrogen. L-alanine-L-alaninol (**52**) was obtained by cleaving the compound from resin **51** (Scheme 2.29).



Reaction conditions: (a) the same conditions as in Scheme 2.19, Scheme 2.20. (b) 5% TFA/DCM, rt, 30 min. (c) trityl chloride resin (1 equiv.), **52** (1.2 equiv.), DIPEA (4 equiv.), DMF, rt, 3 h.

Scheme 2.29 Preparation of resin **53** by re-attaching **52** to resin

Compound **52** was obtained along with small amounts of its TFA ester after cleavage. The mixture, without further purification, was used for re-attachment to trityl resin in the presence of a stronger base, DIPEA. It was found that when pyridine was used, approximately 50% of compound **52** was attached to the resin through the alcohol oxygen atom rather than the amine nitrogen. To evaluate this step, a UV sensitive Dde derivative had to be employed (Scheme 2.30). To track whether compound **52** was attached to the resin via the oxygen or nitrogen, the resin was treated with Dde-OH. Dde-protected L-alanine-L-alaninol was obtained after being cleaved from resin **55**. This material was detected by LC-MS, which implied the presence of resin **51**. The Kaiser test can also be applied to check if there is a free amino group. The purity of resin **53** was 90%, indirectly obtained by checking the purity of resin **55** by LC-UV.



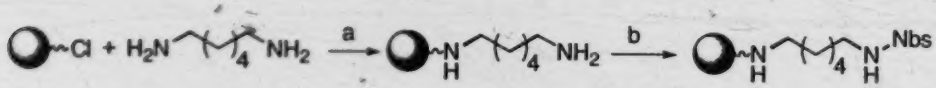
53 **51** **55**

Reaction conditions: (a) resin **53** or **51** (1 equiv.), Dde-OH (1.1 equiv.), Et_3N (2 equiv.), DMF, rt, 30 min.

Scheme 2.30 Evaluation of re-attachment step by Dde protected amine **55**

During elaboration of resin **53**, Mitsunobu reaction conditions were also investigated. The Mitsunobu reaction[57] has been widely applied in organic synthesis since its development. Since diethyl azodicarboxylate (DEAD)-triphenylphosphine (TPP) was applied in the original system, diisopropyl azodicarboxylate (DIAD)-triphenylphosphine (TPP) and 1,1'-(azodicarbonyl)dipiperidine (ADDP)-tributylphosphine (TBP) have also been often used as reagent systems.[58, 59]

As a model study of Mitsunobu reaction conditions, resin **57** was prepared by treatment of 2-nitrobenzenesulfonyl chloride (2-Nbs chloride) with resin bound 1,6-hexadiamine **56** (Scheme 2.31). To obtain resin **56**, large amounts of 1,6- hexadiamine was required to avoid cross linkage. Residue from cleavage of resin **57** gave evidence of mono Nbs protected 1,6-hexadiamine as a single peak in the LC-MS (*m/z* $(M+H)^+$ = 301.2).



56 **57**

Reaction conditions: (a) trityl resin (1 equiv.), 1,6-hexadiamine (10 equiv.), DCM, rt, 2 h. (b) resin **56** (1 equiv.), 2-nitrobenzenesulfonyl chloride (4 equiv.), DIPEA (6 equiv.), DCM, N_2, rt. 3 h.

Scheme 2.31 Preparation of resin **57**, a Mitsunobu reaction substrate

When amino alcohols were used in the literature under Mitsunobu reaction conditions, most cases involved the use of *N*-protected amino alcohols as substrates (Figure 2.5).[23, 26, 59, 60] Bradley and co-workers reported coupling unprotected 6-amino-hexanol and aminophenyl methanols to amidinonapthol on resin (Scheme 2.32).[61] These findings are contrary to the results reported by Jaroszewski[23] in which he found that unprotected aminoalcohols did not provide any of the required compound.

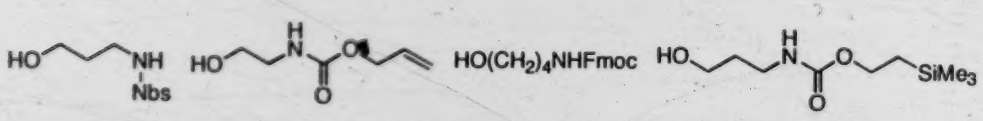
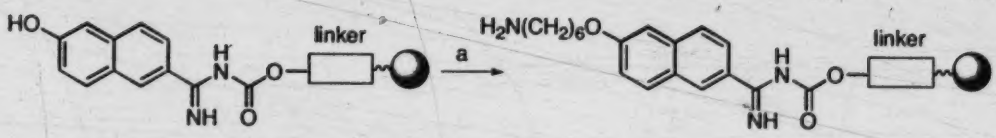


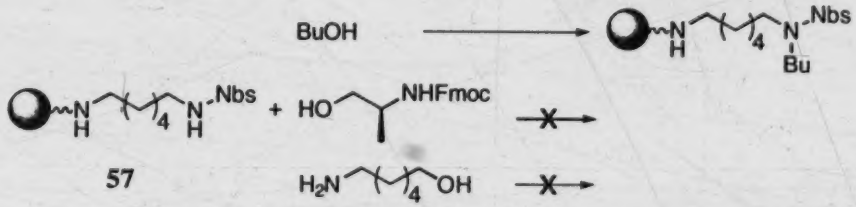
Figure 2.5 Protected amino alcohols used as Mitsunobu reaction substrates



Reaction conditions: (a) 6-aminohexanol, Bu_3P, tetramethylazodicarboxamide, Et_3N, THF, DCM.

Scheme 2.32 Free amino alcohol used as a Mitsunobu reaction substrate by the Bradley group

According to these reports, Fmoc-alaninol, 6-aminohexanol and *n*-butanol were used as substrates in our model study (Scheme 2.33). It was surprising that no desired products were obtained when Fmoc-alaninol and 6-aminohexanol were used, but the control reaction using *n*-butanol proceeded well. For the key example of Fmoc-alaninol, it could be that the methyl group and the protecting Fmoc group make the molecule too hindered for effective coupling. These disappointing results prompted this approach to be abandoned.



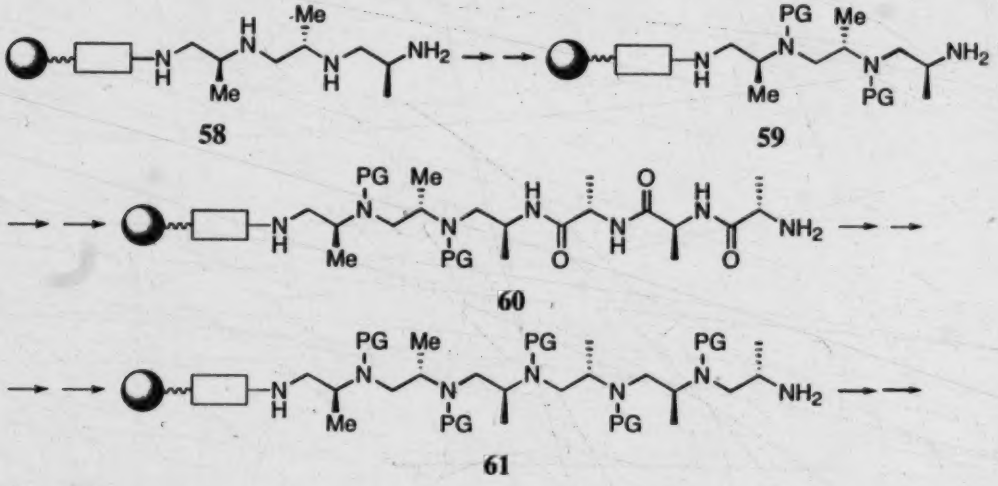
Reaction conditions: resin (1 equiv.), coupling reagents DIAD/Ph_3P or DEAD/Ph_3P, or ADDP/TBP (2 equiv.), THF, rt, 16 h.

Scheme 2.33 Alcohols used as substrates under Mitsunobu conditions

2.3.1.3 Fragment coupling strategy for the synthesis of exo-peptides

When there are more than four amide bonds on the solid supported substrate, the amide carbonyl groups cannot be completely reduced by BH_3-THF. Based on this fact, it could be possible to reduce three amide carbonyl C=O bonds to generate resin bound

polyamine **58** (Scheme 2.34). Then, protection of the resulting free amine nitrogen to give resin **59**, followed by sequential coupling to add another three amino acids could provide resin **60**. Reduction and protection could then give resin **61**. Repeating this sequence could yield long protected amines on solid phase.



Scheme 2.34 Fragment coupling strategy for the synthesis of exo-peptides

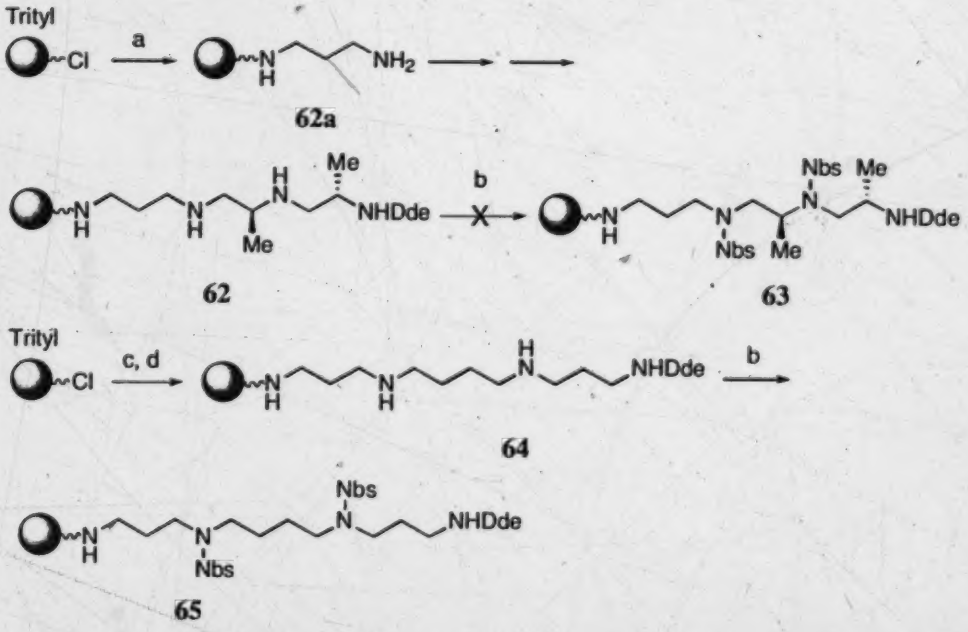
A key objective for this coupling strategy is to find a proper amine protecting group. This group must fully protect secondary amine nitrogens and also be resistant to the harsh reducing conditions (BH_3-THF) and basic conditions (tertiary amines), as well as to be easily deprotected to generate acetylated polyamines.

A review of protecting groups in solid-phase chemistry was outlined by Bradley and coworkers in 2002[62]. Amine protecting groups are divided into two types: base-sensitive or acid-sensitive. i) Base-sensitive groups include Fmoc, 2-(4-nitrophenyl-sulfonyl)ethoxycabonyl (Nsc), Dde, phthalimide, and *o*-nitrobenzene sulfonyl (Nbs) groups. ii) Acid-sensitive groups include Boc, trityl, 2,4-dimethoxybenzyl (DMB), N-diphenylmethyleneamine. iii) other commonly used groups, include: such as 2-trimethyl-silylethoxymethyl (SEM), 2-(trimethylsilyl)-ethoxycarbonyl (Teoc), allyloxycarbonyl, 6-nitroveratryloxycarbonyl (NVOC), 2,2'-bis(2-nitrophenyl) ethoxycarbonyl (diNEOC), benzoyl and benzyl groups.

When considering all the requirements mentioned above, Nbs, trityl and Boc groups could be useful. Although these groups have been widely employed for amine protection in solid-phase synthesis, two potential challenges could be encountered in this project. One is whether these groups can fully protect a polyamine. Another challenge is whether these groups are resistant to the harsh reduction conditions.

In the Mitsunobu coupling strategy, it was found that the 2-nitrobenzene sulfonyl group (Nbs) is compatible with BH_3-THF reducing conditions, so the Nbs group was chosen for our initial study. In considering steric hindrance effects, a parallel control reaction using resin **64** was carried out. Dde protected polyamine resin **62** was prepared using standard procedures.[20, 21] Resin **64** gave mainly the fully protected compound, while in contrast resin **62** only formed the mono protected product (Scheme 2.35).

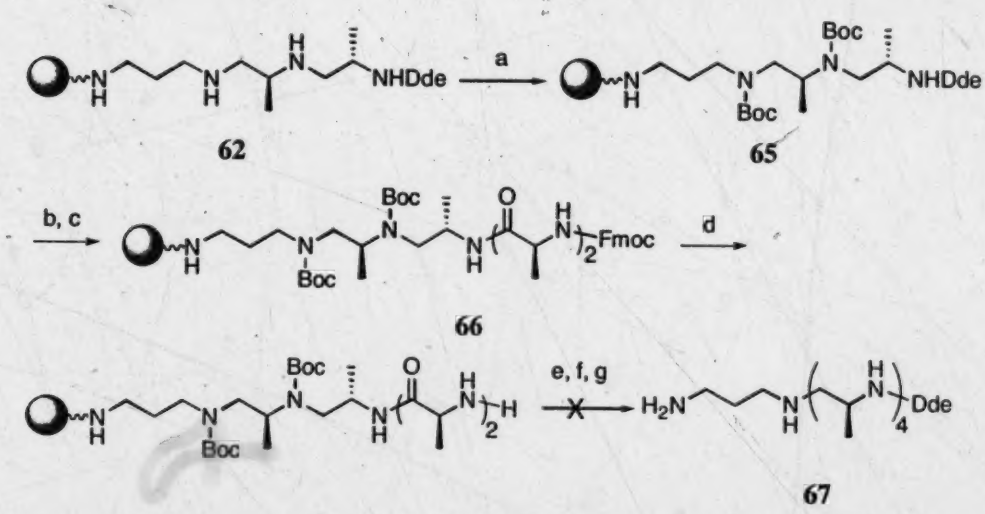
When the trityl group was tested, a similar result was observed.



Reaction conditions: (a) 1,3-propanediamine (100 equiv. respective to resin), DCM, rt, 3 h. (b) Nbs-Cl (8 equiv.), DIPEA (12 equiv.), THF, rt, 16 h. (c) spermine (10 equiv. respective to resin), DCM, rt, 3 h. (d) Dde-OH (1.5 equiv.), DMF, rt, 30 min.

Scheme 2.35 Protection of resin bound polyamine **62** and **64** by Nbs group

Using the Boc protecting group afforded fully protected polyamine **65** from resin **62**. Standard Fmoc AA coupling provided resin **66** (Scheme 2.36). It was hoped that during the reduction step, Boc would be released as a by-product. After the reduction, substantial undesired large peaks (m/z = 317.3+14, 317.3+28) were observed in the LC-MS spectra along with the peak for the desired compound **67** (m/z = 317.3). This was most likely due to over-reduction. The same result was found from resin **64**. The results of polyamine protection with Nbs, Trityl and Boc groups are summarized in Table 2.4.



Reaction conditions: (a) resin **62** (1 equiv.), (t-BuOCO)$_2$O (6 equiv.), DIPEA (6 equiv.), DCM, rt, 40 h. (b) 2% NH_2NH_2/DMF (c) Fmoc AA coupling twice. (d) 20% piperidine/DMF 30 min. (e) (i) 1M BH_3-THF (30 equiv. respective to the resin), 65 °C, 56 h. (ii) piperidine, 65 °C, 16 h. (f) Dde-OH (1.5 equiv.), DMF, rt, 30 min. (g) 33% TFA/DCM, rt, 1 h.

Scheme 2.36 Protection of resin bound amine **62** using the Boc group

Table 2.4 Protecting polyamines with Nbs, trityl and Boc groups

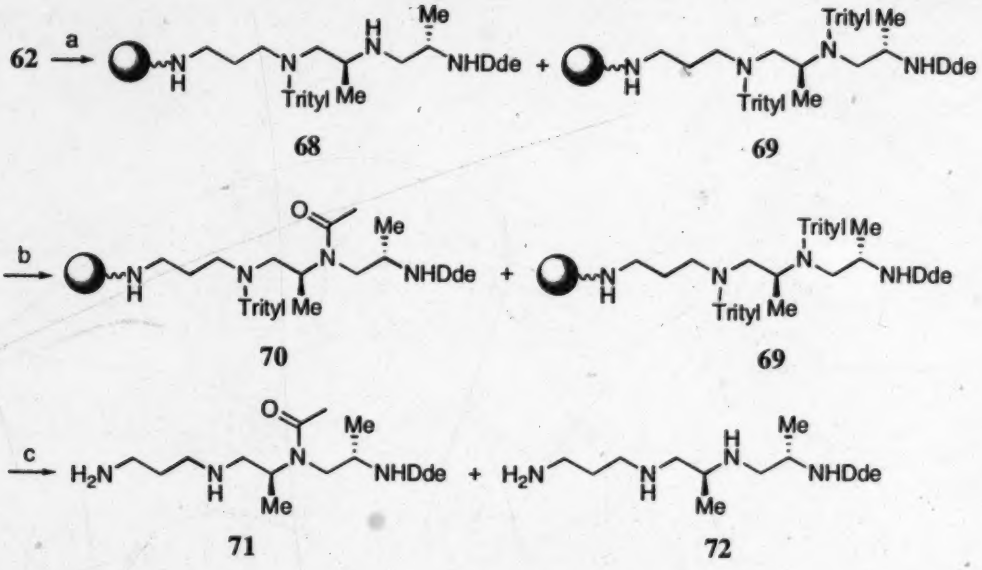
resin	Nbs group	trityl group	Boc group
resin **62**	mainly mono protected, 20% doubly protected	50% doubly protected 50% mono protected	fully protected[i]
resin **64**	60% doubly protected, 30% mono protected	fully protected	fully protected[i]

Note: i) substantial undesired compounds were found after reduction.

From Table 2.4, it can be seen that among Nbs, Trityl and Boc groups, only the Boc group completely protected the resin bound polyamine **62**, but it was not tolerant to the borane-promoted reduction step.

When the Boc or trityl groups were used, direct cleavage with TFA to check the purity by LC-MS was not suitable because the protecting groups will partially or fully detach from the resin due to their acid sensitivity. From previous studies on the acetylation of triamines on solid phase (Scheme 2.20), it was known that the yield of the acetylation was greater than 95%. So, before cleavage, any unprotected free amine nitrogen(s) on the resin was capped by an acetyl group. Acetylated polyamines could be detected by LC-MS to indicate incomplete protection (Scheme 2.37).

For instance, when trityl chloride was used, the mixture of incompletely protected resin **68** and desired resin **69** was subjected to treatment with acetic anhydride to cap any unreacted free amine (Scheme 2.37). The obtained mixture of resin **70** and unreacted resin **69** was treated with 33% TFA to cleave compounds from the resin and also remove trityl groups, providing a mixture of compounds **71** and **72** in a ratio of 1:1 by LC-MS. Unfortunately, compound **71** was obtained from incompletely protected polyamine resin and its observation reflected the low efficiency of the protecting step.

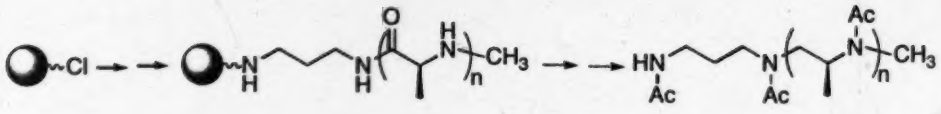


Reaction conditions: (a) resin **62** (1 equiv.), trityl chloride (20 equiv.), DIPEA (50 equiv.), THF, rt, 20 h. (b) Ac_2O (7 equiv.), Et_3N (2 equiv.), DMF, rt, 2 h. (c) 33% TFA/DCM, rt, 30 min.

Scheme 2.37 Indirect method for purity check of trityl protected polyamines

2.3.1.4 Stepwise approach for the synthesis of exo-peptides

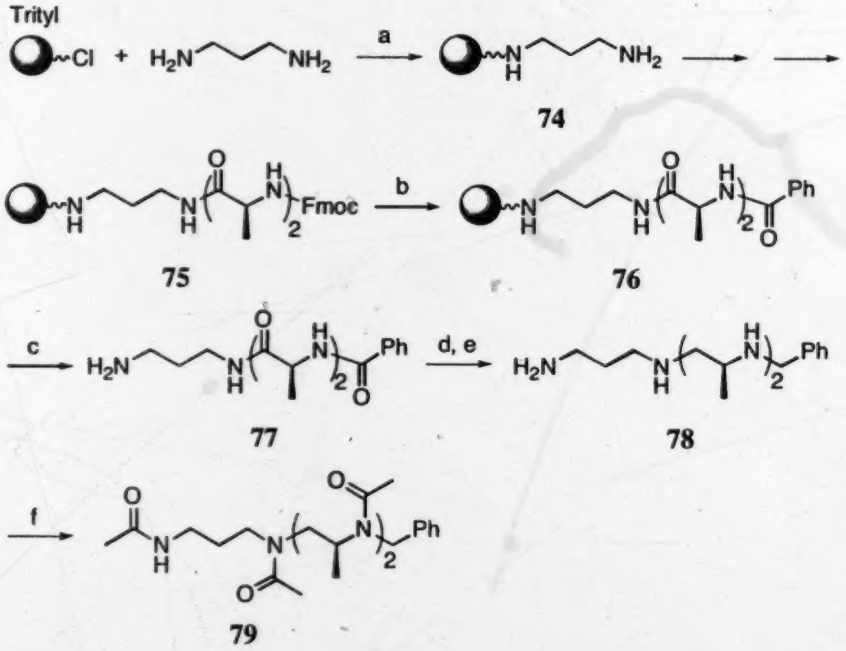
Reduction and acetylation of long peptides in solution phase can provide exo-peptides. Long peptides can be made in solution phase or solid phase. Some long peptides were successfully made in solution phase, but it tends to be a time-consuming effort.[2] Peptide synthesis on solid phase has been well developed. As mentioned in Section 2.1, the advantage of using solid-phase methods was an attractive prospect. As shown in Scheme 2.38, it was hoped that cleavage of resin-bound long peptides **73** followed by reduction and acetylation in solution phase would produce the desired exo-peptides.



73

Scheme 2.38 Plans for stepwise approach for the synthesis of exo-peptides

For this approach, one potential problem could be whether or not the BH_3-THF reduction and acetylation of peptides in solution phase could be carried out in a "one-pot" fashion to provide an acceptable result. Therefore, an initial study of the reduction with BH_3-THF was explored (Scheme 2.39).

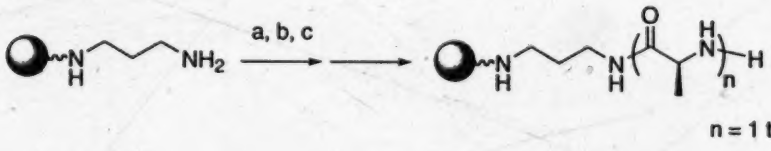


Reaction conditions: (a) (i) 1,3-propanediamine (100 equiv. respective to resin), DCM, rt, 3 h (ii) MeOH, rt, 20 min. (b) (i) 20% piperidine/DMF, rt, 30 min. (ii) benzoyl chloride (4 equiv. respective to the resin), DIPEA (8 equiv.), rt, THF, 3 h. (c) 5% TFA/DCM. (d) 1M BH_3-THF (30 eq.), 65 °C, 2 days. (e) MeOH, reflux, 16 h. (f) Ac_2O (6 eq.), Et_3N (12 equiv.), DMF, 0 °C to rt, 16 h.

Scheme 2.39 Preparation of acetylated triamine **79**

Resin **76** was prepared as shown in Scheme 2.39. After cleavage, the residue was reduced with BH_3-THF followed by work-up upon refluxing with MeOH, then acetylation with acetic anhydride generated the acetylated polyamine **79**. All these three solution steps were operated in "one pot" without purification. LC-MS analysis indicated the presence of the molecular ion for acetylated triamine **79**. However, it was obtained in 75% purity from LC-MS. So, this one-pot reaction was not optimized at this point and no further chromatography of **79** was done.

Following the success of the above "one-pot" approach, the remainder of this manuscript regarding the stepwise approach will focus on construction of long peptides on solid phase. Resin bound peptides were assembled by repeating standard Fmoc-AA coupling condition (Scheme 2.40).



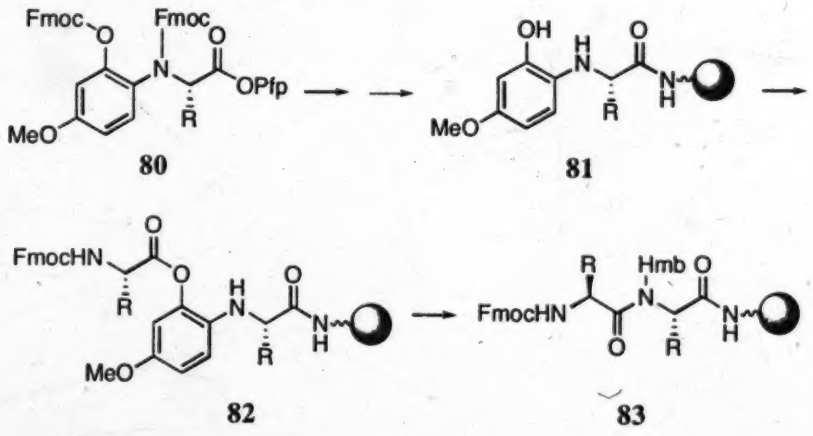
Reaction conditions: (a) Fmoc-L-alanine (4 equiv.) HBTU (4 equiv.), HOBt (4 equiv.), DIPEA (8 equiv.), DMF, rt, 3 h. (b) 20% piperidine/DMF 30 min. (c) repeat step (a), (b).

Scheme 2.40 General stepwise procedure for the synthesis of resin bound peptides

All coupling and deprotection steps were monitored by the Kaiser test and by LC-MS analysis. It was found that when up to five alanine units were employed, all coupling and Fmoc removal steps worked well. Incomplete coupling occurred when the peptide chain was extended to 6-alanine units. After coupling, the pink color of the resin was observed by a Kaiser test, which indicated the presence of unreacted free amines. Double coupling did not force the reaction to completion, instead, undesired peptide residues containing 5- and 7-alanine units were detected by LC-MS. This phenomenon is called aggregation or "difficult sequences," which is a major problem during long peptide synthesis. This is due to the intra- or interchain interactions caused by hydrogen bonding

and hydrophobic forces. Shepard and co-workers[30] extensively studied the aggregation of different homopeptides and stated that different amino acids show different aptitudes for aggregation and the aggregation occurs from the 12-unit polyproline peptide and 6-unit polyalanine peptide. To overcome this problem, researchers have found several ways to reduce aggregation. For example, Pugh and coworkers[63] stated that good resin swelling properties, low substitution and better uniformity of beads can be helpful. Longer coupling times, double coupling, acyl capping of free amine residues, the use of chaotropic salts (0.8 M LiCl in DMF) and "magic mixture" solvent (DCM/DMF/NMP (1:1:1) with 1% triton x 100 and 2M ethylenecarbonate) [64, 65] were all investigated, but, no improvement was observed.

The effects of intra- or interchain interactions by hydrogen bonding, causing aggregation, can be reduced when other residues are inserted into the chain to mitigate these interactions. Two types of amino derivatives have been designed for this purpose. One is a 2-hydroxy-4-methoxybenzyl (Hmb) amino acids (Scheme 2.41),[66] and the other is an oxazolidine dipeptide derivative, which incorporates a pseudoproline residue derived from either serine or threonine.[67]



Scheme 2.41 The use of Hmb amino acids in the synthesis of peptides

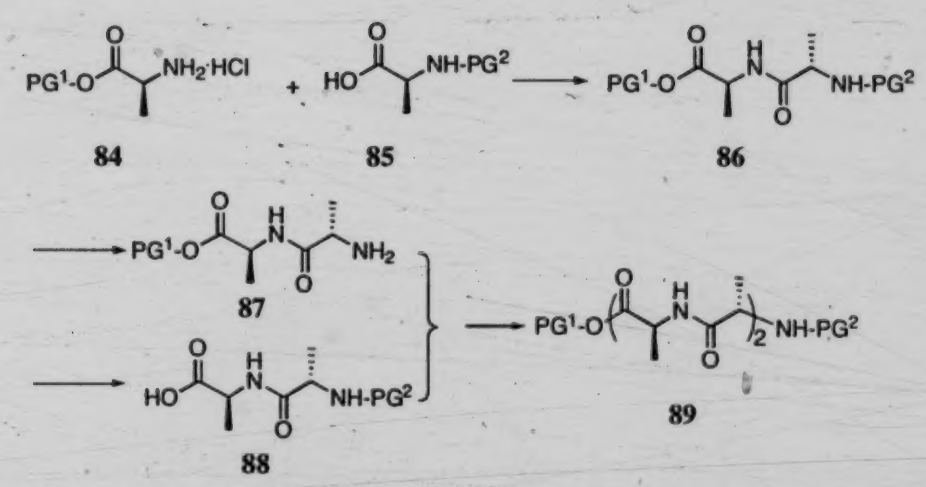
The Hmb strategy (Scheme 2.41) involves coupling Hmb amino acids to resin bound peptides, followed by Fmoc group removal, to provide resin bound Hmb protected

peptide **81**. Coupling with an Fmoc amino acid generated resin **82** as an intermediate, which underwent intramolecular O→N acyl transfer to yield the desired tertiary amide **83**. Due to the use of Hmb, intra- or interchain interactions were decreased, therefore, the coupling reaction went to completion. The Hmb amino acids needed to be incorporated at every sixth to eighth residue. The Hmb group can be cleaved at the last step when the compound is released from the resin.

This strategy was successfully used for synthesizing a 23-residue peptide, part of the amino acid sequence of the influenza virus hemagglutinin.[68] A 20-residue peptide, human $\alpha_{1E\text{-}3}$ calcium channel subunit residues 985-1004 was also synthesized.[69] The Hmb strategy could be a promising strategy for this project but it would be advantageous to find a more general and less expensive method. One reason is that only a few Hmb amino acids are commercially available. The other reason is its eventual cost (the price of L-alanine Hmb derivative, *N*-α-Fmoc-*N*-α-(2-Fmoc-oxy-4-methoxybenzyl)-L-alanine pentafluorophenyl ester, was 353 US$/gram, Novabiochem, 2002/3 catalog) when multigram quantities or more of the starting material are required. This is because in solid phase synthesis, a large excess of reagents is demanded.

2.3.2 The synthesis of exo-peptides through solution phase

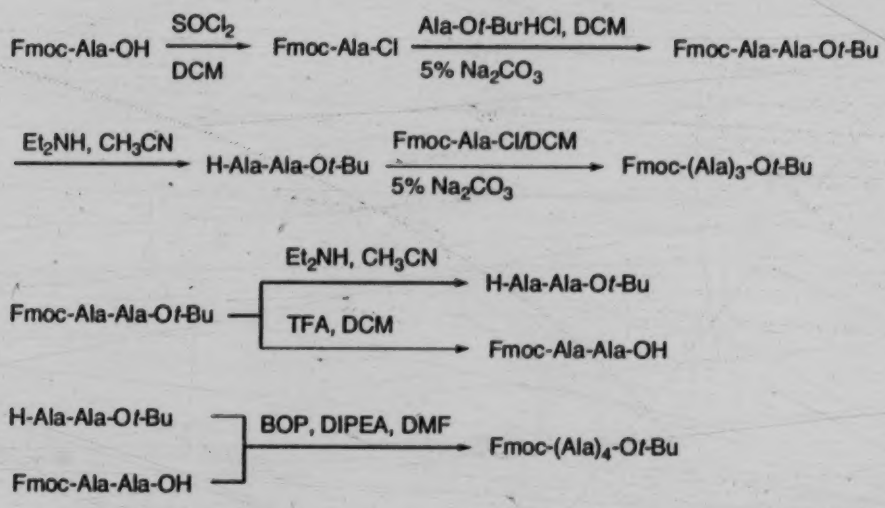
Although synthesis of peptides in solution phase is time consuming, segment coupling strategy in solution phase to make long peptides has been applied by many groups. An initial plan for new strategy is shown in Scheme 2.42. Two different groups were required to protect L-alanine on both the amine and carboxyl sides. Coupling the protected L-alanine **84** and **85** would provide dipeptide **86**. Under acidic or basic conditions, one of the protecting groups could selectively be removed, thus providing dipeptides **87** and **88** respectively. Continuing on this coupling and deprotection sequence, long peptides could be prepared in solution phase. Proper protecting groups are crucial for this segment coupling strategy, and as such, they should be stable under amide bond formation conditions and be selectively removed.



Scheme 2.42 General segment approach for peptide synthesis in solution phase

Bodanszky has summarized protecting groups for peptide synthesis. [3] Cameron [70] described the synthesis of tetra-alanine and hexa-alanine by using Fmoc-alanine and Ala-O*t*-Bu (Scheme 2.43). The advantage of having the *t*-Bu and Fmoc group is that *t*-Bu is deprotected under acidic conditions and Fmoc is deprotected under basic conditions. Thus, both groups are widely used for peptide synthesis.

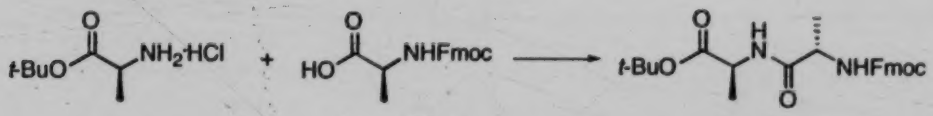
Cameron's segment strategy met the project requirements. Following this concept, if every step worked well, long peptides could be constructed in solution phase. Therefore, this strategy was applied for the construction of polyalanine intermediates for accessing the corresponding exo-peptides.



Scheme 2.43 Fragment approach of synthesis of polyalanine peptides by the Cameron group

2.3.2.1 Synthesis of dipeptide 90

Dipeptide Fmoc-Ala-Ala-O*t*-Bu (**90**) was prepared in 92-95% yield without any problems, following the literature procedure[3] (Scheme 2.44). DCC and HOBt were used as coupling reagents. The purification was performed by flash column chromatography for small scale and recrystallization for large scale.



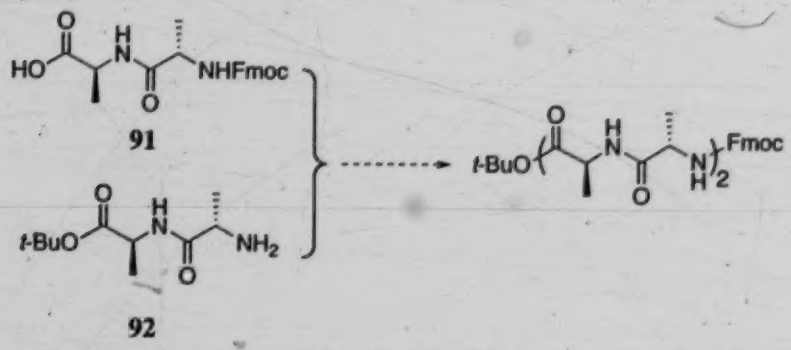
122

Reaction conditions: L-alanine *t*-butyl ester hydrochloride (1 equiv.), *N*-ethylmorpholine (1 equiv.), HOBt (1 equiv.), DCC (1.05 equiv.), Fmoc-L-alanine (1 equiv.), THF, 0 °C, 1 h, then rt, 1 h.

Scheme 2.44 Preparation of Fmoc-Ala-Ala-O*t*-Bu

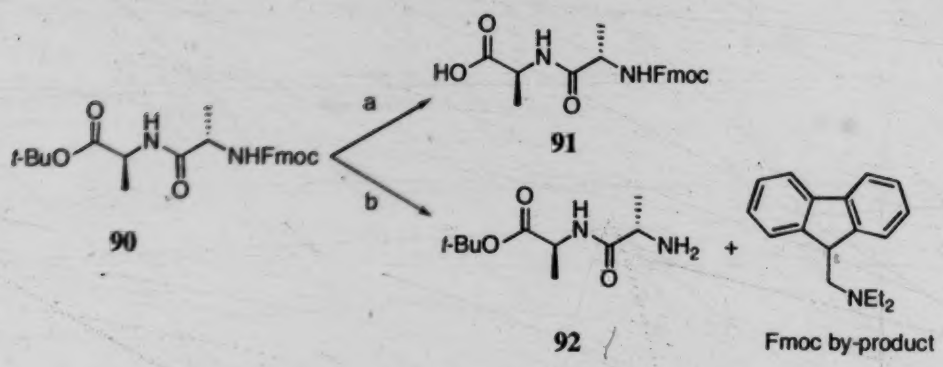
2.3.2.2 Synthesis of tripeptide 93

According to the initial plan, once dipeptide Fmoc-Ala-Ala-O*t*-Bu (**90**) was synthesized, segment coupling would be applied for the synthesis of a tetra-alanine peptide (Scheme 2.45) from semi protected dipeptides Fmoc-Ala-Ala-OH (**91**) and Ala-Ala-O*t*-Bu (**92**)



Scheme 2.45 Initial plan for the synthesis of Fmoc-$(Ala)_4$-O*t*-Bu

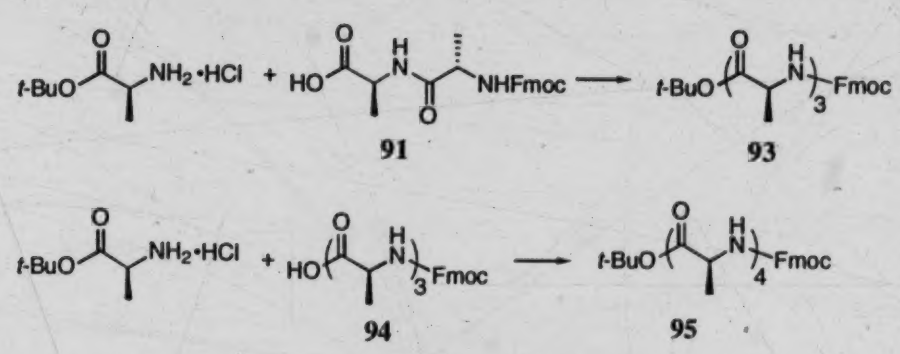
Semi protected dipeptide **91** was obtained as a white solid after a quantitative deprotection of the *t*-Bu with TFA/ triethyl silane[71] or 1M HCl/HOAc[72]. This deprotection step was monitored by TLC. It was found that the TFA/triethylsilane procedure took more than one day for the reaction to go to completion, compared to eight hours with the HCl/HOAc method. Hence, the HCl/HOAc method was used as a standard procedure to remove *t*-Bu groups in this project. Simple stirring of the solution of Fmoc-Ala-Ala-O*t*-Bu in 1M HCl/HOAc at room temperature, followed by evaporation under reduced pressure provided pure compound **91**. This result was confirmed by ^{1}H-NMR, IR, HR-MS and LC-MS analysis.



Reaction conditions: (a) dipeptide **90**, 1M HCl/HOAc, rt, 8 h. (b) dipeptide **90** (1 mmol), Et_2NH (1 mL), THF, rt, 2 h.

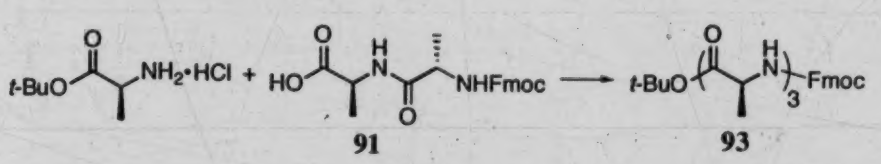
Scheme 2.46 Selective deprotection of Fmoc or *t*-Bu groups

Semi-protected dipeptide Ala-Ala-O*t*-Bu (**92**) was obtained by treatment of dipeptide **90** with diethylamine or piperidine (Scheme 2.46). Compound **92** was obtained as an oil and was of a highly polar nature, which made purification difficult. If products are solids, washing with diethyl ether is a common method to remove the Fmoc by-product dibenzofulvene adduct and excess base. Excess secondary amine must be removed because remaining secondary amine will affect the subsequent coupling reaction because remaining secondary amine will deprotect the Fmoc group of Fmoc-Ala-Ala-OH (**91**) in next coupling step. Free amines could be purified by ion-exchange column chromatography, but the acid sensitive *t*-Bu group made this method unpractical. An alternative for overcoming this problem could be to halt purification until the semi-protected peptide moieties are solid, such as when they contain four or five Ala units. So, we modified the strategy to a stepwise synthesis of the tetrapeptide Fmoc-$(Ala)_4$-O*t*-Bu (Scheme 2.47).



Scheme 2.47 Stepwise approach for the synthesis of Fmoc-$(Ala)_4$-O*t*-Bu

Thus, the tripeptide Fmoc-$(Ala)_3$-O*t*-Bu (**93**) was obtained in 95% yield after purification by column chromatography (Scheme 2.48), which was characterized by IR, ^{1}H NMR, ^{13}C NMR, HR-MS and LC-MS.

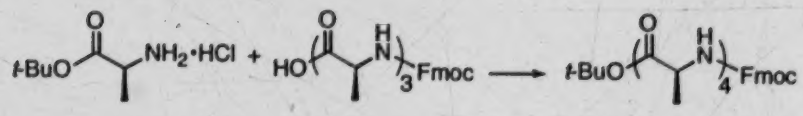


Reaction conditions: L-alanine *t*-butyl ester hydrochloride (1equiv.), Fmoc-Ala-Ala-OH (1 equiv.), HOBt (1equiv.), DCC (1.05 equiv.), *N*-ethylmorpholine (1 equiv.), THF, 0 °C, 1 h, then rt, 1 h.

Scheme 2.48 Preparation of Fmoc-$(Ala)_3$-O*t*-Bu tripeptide

2.3.2.3 Synthesis of tetrapeptide 95

As expected, treatment of tripeptide **93** with 1M HCl/HOAc generated the carboxylic acid moiety Fmoc-$(Ala)_3$-OH (**94**) in quantitative yield. DCC was utilized as an activating reagent to synthesize the tetrapeptide Fmoc-$(Ala)_4$-O*t*-Bu (**95**) (Scheme 2.49).



94 95

Reaction conditions: L-alanine *t*-butyl ester hydrochloride (1equiv.), Fmoc-$(Ala)_3$-OH (1 equiv.), HOBt (1equiv.), DCC (1.05 equiv.), *N*-ethylmorpholine (1 equiv.), THF, 0 °C, 1 h, then rt, 1 h.

Scheme 2.49 Preparation of tetrapeptide **95** using DCC/HOBt

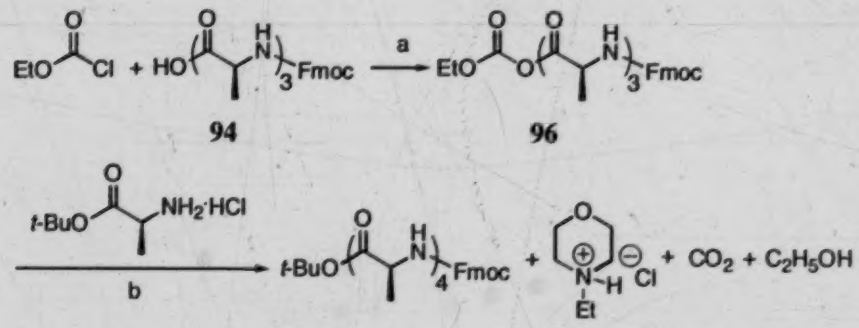
The same procedure used in the synthesis of dipeptide **90** generated the tetrapeptide Fmoc-$(Ala)_4$-O*t*-Bu (**95**), but purification was problematic. After the reaction, the DCC by-product, *N,N'*-dicyclohexylurea, was filtered out. Evaporation of solvent provided a white product, tetrapeptide **95**, only in a 28% yield accompanied a small amount of the DCC by-product. The major portion of compound **95** was found to be filtered out along with the DCC by-product. The low solubility of *N,N'*-dicyclohexylurea and tetrapeptide **95** caused the problem. A solubility test showed that tetrapeptide **95** only partially dissolved in common solvents, such as THF, EtOAc, hexane, and DCM, and was insoluble in water. Hence, it was difficult to purify **95** by flash column chromatography. Recrystallization did not completely remove *N,N'*-dicyclohexylurea.

This purification problem has been mentioned previously in peptide synthesis.[8, 73] To handle this problem, other carbodiimide reagents were developed, such as 1,3-diisopropyl carbodiimide (DIC) and 1-cyclohexyl-3-isopropyl carbodiimide (CIC). Ureas from DIC, CIC are relatively soluble in DCM compared to the urea obtained from DCC. Their respective solubility in DCM are 30, 5.2 and 1.5 g/L[8]. It was hoped that the solubility difference of ureas would be helpful in the removal of impurities by recrystalization. When DIC was employed, no improvement was observed. Acid-base extraction of Fmoc-$(Ala)_4$-OH did not work either.

Due to the purification problems, it was decided to avoid using carbodiimide reagents for the peptide couplings. Since the product **95** has a low solubility in common organic solvents and insoluble in water, a search was conducted for a reagent in which

their by-products can be washed away with water and the product can be precipitated. The mixed anhydride method[2] met this requirement (Scheme 2.2). After the reaction, only water-soluble ammonia salts and alcohols are formed as by-products.

Following the literature procedure[3], treatment of tripeptide **94** with ethyl chlorocarbonate generated the mixed anhydride intermediate **96**, which was immediately reacted with L-alanine *t*-butyl ester providing tetrapeptide **95** (Scheme 2.50). After the reaction, DMF was evaporated and the residue re-dissolved in a minimal amount of DMF. Diluting the DMF solution with water (v/v=1:10) provided a white precipitate. Washing the precipitate with water generated the highly pure tetrapeptide **95** in 81% yield. The latter was characterized by IR, ^{1}H-NMR, ^{13}C-NMR, HR-MS and LC-MS.



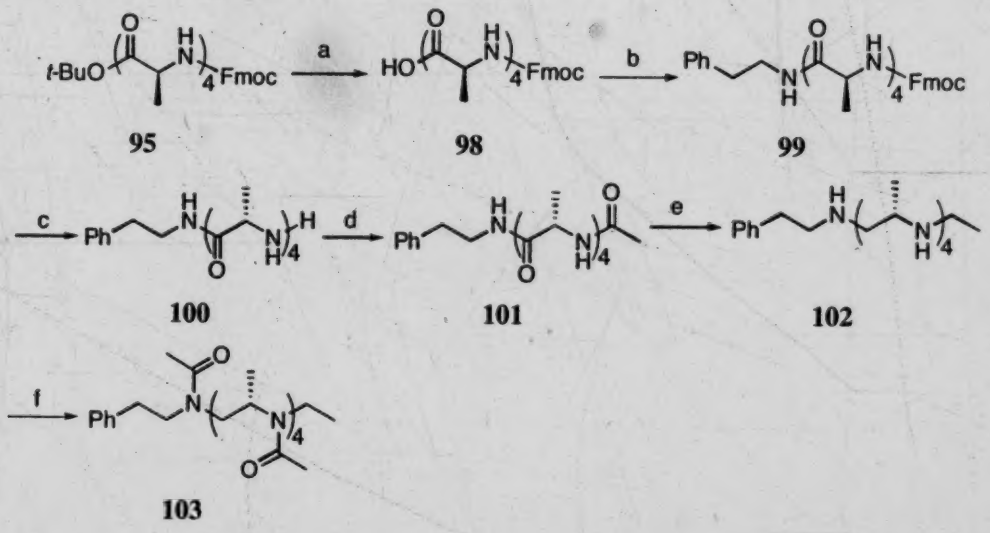
Reaction conditions: (a) Fmoc-(Ala)$_3$-OH (**94**) (1 equiv.), *N*-ethylmorpholine (1 equiv.), ethyl chlorocarbonate (1.05 equiv.), -15 °C, 1 min. (b) L-alanine *t*-butyl ester hydrochloride (1 equiv.), *N*-ethylmorpholine (1 equiv.), -15 °C to rt, overnight.

Scheme 2.50 Preparation of tetrapeptide **95** through mixed anhydride intermediate **96**

2.3.2.4 Model study of acetyl pentaamine

Like the model study in Section 2.3.1.4, once tetrapeptide **95** was in hand, a "one pot" reaction was carried out to make sure that reduction, work-up and acetylation steps in solution phase worked well. A short exo-peptide **103** was prepared as shown in

Scheme 2.51. In order to use LC-MS chromatography to monitor reactions, the carboxyl side of **98** was capped with 2-phenylethylamine providing compound **99** in 69% yield. The solubility of compound **99** was quite low in DCM, THF, DMSO and DMF. Deprotection of the Fmoc group using Et_2NH, followed by acetylation provided compound **101** in 65% yield. Reduction of polyamine **101** with BH_3-THF, followed by work-up upon refluxing with MeOH, and finally acetylation in a "one pot" fashion generated short exo-peptide **103**. LC-MS analysis indicated the presence of the molecular ion for acetylated pentamine **103**. However, it was obtained in 77% purity in LC-MS. So, this one-pot reaction was not optimized at this point and no further chromatography of **79** was done.

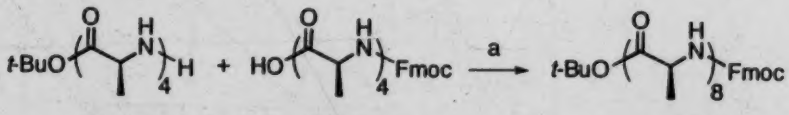


Reaction conditions: (a) 1M HCl/HOAc, rt, 16 h. (b) (i) Fmoc-$(Ala)_4$-OH (1 equiv.), ethyl chlorocarbonate (1.05 equiv.), *N*-ethylmorpholine (1 equiv.), -15 °C, 1 min. (ii) 2-phenyl ethylamine hydrochloride (1 equiv.), *N*-ethylmorpholine (1 equiv.), -15 °C to rt, overnight. (c) compound **99** (1 mmol), Et_2NH (1 mL), DMF, rt, 3 h. (d) compound **100** (1 equiv.), Ac_2O (4 equiv.), Et_3N (6 equiv.) (e) (i) compound **101** (1 equiv.), 1M BH_3-THF (50 equiv.), THF, 65 °C, 3 days. (ii) MeOH, reflux, 16 h. (f) compound **102** (1equiv.), Ac_2O (10 equiv.), Et_3N (20 equiv.).

Scheme 2.51 Preparation of short exo-peptide **103**

2.3.2.5 Synthesis of octapeptide 137

Octapeptide **106** could be generated by segment coupling between semi-protected tetrapeptide (Ala)$_4$-O*t*-Bu (**104**) and Fmoc-(Ala)$_4$-OH (**105**). Compound **104** was obtained in 78% yield as a white solid by treating Fmoc-(Ala)$_4$-O*t*-Bu (**95**) with diethylamine. Fmoc-(Ala)$_4$-OH (**105**) was obtained quantitatively using the HCl/HOAc procedure to remove the *t*-Bu group.



104 **105** **106**

Reaction conditions: (a) Fmoc-(Ala)$_4$-OH (1 equiv.), ethyl chlorocarbonate (1.05 equiv.), *N*-ethyl morpholine (1 equiv.), -15 °C, 1 min. (b) H-(Ala)$_4$-O*t*-Bu (1equiv.), *N*-ethyl morpholine (1 equiv.), -15 °C to rt, overnight.

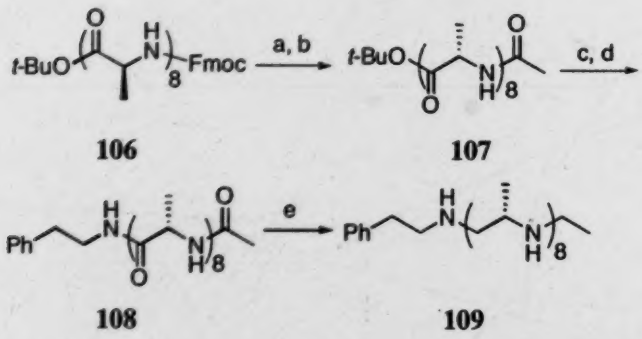
Scheme 2.52 Preparation of octapeptide **106**

Using the mixed anhydride method, with ethyl chlorocarbonate as an activating reagent, octapeptide Fmoc-(Ala)$_8$-O*t*-Bu (**106**) was obtained in 62% yield. The low yield could be due to a lower concentration of coupling substrates **104** and **105**. In order to dissolve these substrates, large amounts of DMF had to be used (10 ml DMF/100 mg), so, trace water from DMF could significantly destroy the activating reagent, ethyl chlorocarbonate. To improve the yield, the coupling reagent *o*-phenylene phosphorochloridite was tried. Anderson[74] applied this reagent to generate a 9-unit peptide. According to their procedure, *o*-phenylene phosphorochloridite was added to a solution of the two coupling segments and Et_3N then stirred overnight. After the reaction, the compound **106** was precipitated by diluting the reaction solution with water, but only a 39% yield was observed. Although there are many coupling reagents that could be used to try to improve the yield of this step, no further investigation in optimizing the yields

was undertaken. This was due to the low solubility of octapeptide **106** which prompted this route to be abandoned.

Octapeptide **106** hardly dissolved in DMF, DMSO and was almost insoluble in water and common organic solvents, such as MeOH, EtOAc and THF. To evaluate its purity, LC-MS was utilized first. It was surprising that no product signal was found in ESMS spectrometry, unlike the starting material Fmoc-$(Ala)_4$-OH. It was realized that maybe the compound **106** was filtered out before injection. To purify the octapeptide, extraction of compound **106** in MeOH was performed three times, and around 15%, 2% and 1% of solid was extracted out respectively. After extraction, the Fmoc-$(Ala)_4$OH signal in LC-MS was lower and compound **106** was still not found. HR-MS spectrometry showed the presence of octapeptide **106** (cal. 887.42738, found: 887.42762). ^{1}H-NMR was also attempted. Heating and sonication had to be used to accelerate the dissolution of the sample in DMSO-d_6. In ^{1}H-NMR, broad signals were recorded. The signal pattern matched spectra obtained for Fmoc-$(Ala)_8$-O*t*-Bu, but the proton integration ratio slightly deviated. ^{13}C-NMR signals were low and contained many overlaps, such as methyl groups and amide carbonyl groups. Due to the weak signals in the NMR spectra and no corresponding signal in LC-MS spectrometry, it was difficult to characterize this octapeptide Fmoc-$(Ala)_8$O*t*-Bu (**106**). A similar situation of evaluating polypeptides occurred in the synthesis of an 81-unit heteropeptide by Hofmann.[13] Hofmann stated that "elucidation of the homogeneity of complex synthetic peptides is a difficult task. Homogeneity can only be demonstrated in a negative sense, i. e., by failure to find imperfections by using various analytical techniques. …". Hofmann relied on amino acid composition analysis to evaluate the purity of fragments, but amino acid composition analysis is not good for homopeptides. Deprotecting the Fmoc and *t*-Bu group of **106** did not increase the solubility. It was thought that after the polypeptides were reduced, the resulting polyamines would be more soluble. Therefore, polyamine **109** was prepared from Fmoc-$(Ala)_8$-O*t*-Bu (Scheme 2.53). Due to the low solubility, it was not possible to monitor the transformation of compound **106** to **108** by TLC and LC-MS. Although signals in the ^{1}H-NMR spectra were weak, compound **109** showed signals corresponding to acetyl, methyl and phenyl groups. After reduction and work-up, polyamine **109** was

obtained (m/z $(M+H)^+$ = 606.5), but only showed 50% purity in the LC-MS chromatogram.

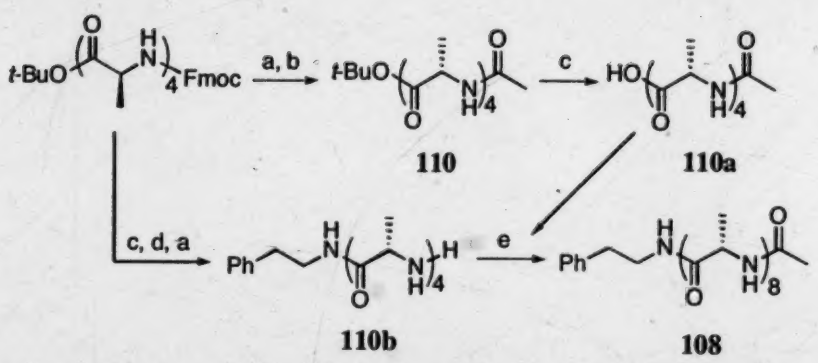


Reaction conditions: (a) **106** (1 mmol), Et_2NH (1 mL), DMF, rt, 3 h. (b) Ac_2O (4 equiv.), Et_3N (6 equiv.). (c) 1M HCl/HOAc, rt, 16 h. (d) (i) acetyl-$(Ala)_8$-OH (**107**) (1equiv.), *N*-ethylmorpholine (1equiv.), ethyl chlorocarbonate (1.05 equiv.), -15 °C, 1 min. (ii) *N*-ethylmorpholine (1 equiv.), 2-phenyl ethylamine hydrochloride (2 equiv.), -15 °C to rt, overnight. (e) (i) 1M BH_3-THF (50 equiv.), THF, 65 °C, 4 days. (ii) MeOH, reflux, 16 h.

Scheme 2.53 Preparation of polyamine **109**

It was difficult to monitor reactions going from compound **106** to compound **109**. Segment coupling is an alternative method to construct compound **108** (Scheme 2. 54).

Although octaamine **109** was detected in HR-MS, and it could be acetylated and further purified, it was felt that it would be extremely difficult to synthesize longer exo-peptides. This was due to solubility problems and difficulties in monitoring reactions. At this point, long exo-peptides were not synthesized, but the challenges met in this project should be pointed out: 1) in solid-phase approach, the C-O bond in the resin linkage was not tolerant to 1M BH_3-THF, which caused the Mitsunobu coupling strategy to fail. 2) peptide aggregation in the solid-phase linear strategy. 3) the failure to find proper protecting groups for segment coupling. 4) low solubility issues in the solution phase.



Reaction conditions: (a) **95** (1.0 mmol), Et_2NH (1.0 mL), DMF, rt, 3 h. (b) Ac_2O (4 equiv.), Et_3N (6 equiv.), rt, overnight. (c) 1M HCl/HOAc, rt, 16 h. (d) (i) Fmoc-$(Ala)_4$-OH (1 equiv.), ethyl chlorocarbonate (1.05 equiv.), *N*-ethymorpholine (1equiv.), -15 °C, 1 min. (ii) 2-phenyl ethylamine hydrochloride (1 equiv.), *N*-ethyl morpholine (1.0 equiv.), -15 °C to rt, overnight. (e) (i) **110a** (1.0 equiv.), *N*-ethyl morpholine (1.0 equiv.), ethyl chlorocarbonate (1.05 equiv.), -15 °C, 1 min. (ii) **110b** (1.0 equiv.), *N*-ethylmorpholine (1.0 equiv.), -15 °C to rt, overnight.

Scheme 2.54 Alternative route for the preparation of peptide **108**

2.4 Summary

Design, synthesis and studies of unnatural biopolymers are important ways for humans to understand the natural world, and eventually provide in the benefits of human diseases treatment, such as HIV and cancer therapies. Based on the concept of forming secondary structures and the conformational behaviors of other unnatural biopolymers such as β-peptides and β-peptoids, exo-peptides were designed. To synthesize exo-peptides, two synthetic pathways were attempted. The first one involved the extension of polyamines through Mitsunobu reactions, and the second one was the assembly of long peptides followed by reduction and acetylation. Solid phase and solution phase techniques were utilized. In the solid phase methods, it was found that the C-O ether bond utilized as a linker was not tolerant to the BH_3-THF reduction, and Fmoc-L-alaninol is too hindered to couple with 2-nitrobenzenesulfonamides on solid support. These problems resulted in the failure of Mitsunobu reactions. It was also found that aggregation occurred during peptide bond extension on the solid support, and the low solubility of model

polyalanine peptides caused problems during the formation of peptide bonds through the segment coupling strategy in solution phase. Among different protecting groups, only the Boc group afforded the fully protected secondary polyamines on solid phase.

2.5 Experimental Section

2.5.1 General

DCM, toluene and MeOH were distilled over CaH_2. THF was dried and distilled over sodium/benzophenone ketyl. Ac_2O, pyridine, and acetone were purified and dried according to literature procedures.[75] All reagents are commercially available and were used without purification. Fmoc-L-alanine, L-alanine *t*-butyl ester hydrochloride, HBTU, HOBt and Dde-OH were purchased from Novabiochem (California, US). Polypropylene vessels were purchased from Bio-Rad Laboratories (California, US). All resins from Novabiochem (California, US), or Rapp-Polymere (Tübingen, Germany) or Matrix Innovation Inc. (Montral, Canada). Other chemicals from Aldrich.

LC-MS spectra were recorded on an Agilent HPLC 1100 series (Agilent). UV spectra were recorded on a Hewelett Packard 8450A diode array spectrophotometer. IR spectra (cast film) were recorded on a Nicolet Magna-IR™ spectrometer 750; IR spectra (microfilm) were recorded on a Nic-Plan™ IR microscope. ^{1}H NMR spectra were recorded on Varian 300 (300 MHz), or Varian 400 (400 MHz) or Varian 500 (500 MHz). ^{13}C NMR spectra were recorded on Varian 400 (100 MHz) or Varian 500 (125 MHz) (Varian INOVA). Chemical shifts are reported in ppm from tetramethylsilane using solvent resonances as internal standards. High resolution mass spectra were recorded on ZabSpec oaTOF (Micromass) or Applied BioSystems Marinear Biospectrometry Workstation (Applied Biosystem). Elemental analyses were measured on a CHNS-O EA1108 elemental analyzer (Cario Erba Instruments). Melting points were measured on a Gallenkamp melting point apparatus and are uncorrected.

2.5.1.1 Measurement of new loading of resin by UV

According to the literature procedure, [76] asccurately weighed resin (nominally 25 mg) is placed in a vial with a stir bar, followed by addition of 1.00 mL piperidine/DMF

(v/v = 3:7). The resulting suspension is gently stirred for 30 min at room temperature, followed by filtration of the solution into a 25.00 mL volumetric flask through a pipette with a glass wool plug. The resin is washed with 13.0 mL MeOH, and the wash solution is combined to the volumetric flask, then diluted to 25.00 mL with mixed solvent (MeOH: piperidine : DMF = 13 : 3 :7). 1.00 mL solution from above 25.00 mL volumetric flask is diluted with mixed solvent (MeOH: piperidine : DMF = 13 : 3 :7) to 25.00 mL for UV measurement at 301 nm. The mixed solvent (MeOH: piperidine : DMF = 13 : 3 :7) is used as a blank sample.

$$\text{loading level (mmol/g)} = \frac{A}{W} \times 80.13$$

A: UV absorbance at 301nm
W: mass of resin (mg)

2.5.1.2 HPLC-LC-MS conditions

Reverse phase gradient elution method 1:

Column: Zobax SB-C8 column (4.6 x 50 mm, 3.5 μm)

Mobile phase: A: acetonitrile (0.1% TFA), B: water (0.1% TFA)

Gradient: 5 to 85% A in 5 min. maintained 85% A for 7 min. 85 to 5% A in 3 min.

Flow rate: 0.7 mL/min; Injection: 2 μl; Temp: 25 °C

Detector: UV DAD at 210 and 254 nm, MSD.

Reverse phase gradient elution method 2: The same as reverse phase gradient elution method 1, except flow rate: 0.5 mL/min.

Reverse phase gradient elution method 3: The same as reverse phase gradient elution method 1, except gradient.

Gradient: 5 to 85% A in 12 min., 85 to 70% A in 2 min., 70 to 5% A in 2 min.

2.5.1.3 General procedure for the preparation of peptides through Fmoc-amino acid coupling method

Dry resin in polypropylene vessel (PP vessel) was swelled in dry DCM for 10 min, followed washing with dry solvents DCM, THF, and DMF twice each, and then the resin

was suspended in dry DMF (10 mL per gram resin). To this suspension was added Fmoc-amino acid (4 equiv.), HBTU (4 equiv.) and HOBt (4 equiv.), and the mixture was vortexed for 3 min until all reagents had dissolved. This was followed by addition of DIPEA (8 equiv.) in one portion. The PP vessel was capped sealed, and vortexed for 3 h at room temperature. The resultant suspension was drained and washed with DMF, THF, MeOH, and DCM three times each. The resin gave negative Kaiser test. The Fmoc group was removed by suspending the resin with 20% piperidine in DMF, then vortexed for 10 min, and the solvents were drained. The resin was subjected to treatment with 20% piperidine in DMF, vortexed 30 min followed by wash with DMF, THF, MeOH and DCM three times each.

2.5.1.4 General procedures to cleavage of compounds from trityl resin

Cleavage of compounds for LC-MS analysis:

A small amount (~1 mg) of resin and 1 mL 5% TFA/DCM was placed inside a vial with a stir bar. The suspension was stirred at room temperature for 30 min. The resin was filtered through a glass wool plug in a glass pipette. The resin on top of the plug was rinsed with 0.5 mL MeOH. After evaporation of organic solution, the residue was dissolved in 0.5 mL MeOH (HPLC grade), then filtered through a 0.2 μm filter tip.

Cleavage compounds (large scale):

The resin was placed inside a clean fritted funnel with 3-way valves. 5% TFA/DCM (10 mL per gram resin) was then added. The funnel was shaken for 30 min. The resulting solution was drained into a round-bottom flask. The treatment of the resin with 5% TFA/DCM was repeated twice. The combined organic solution was evaporated under reduced pressure. The resultant residue dried *in vacuo* overnight.

2.5.1.5 General procedure of reduction of resin bound peptides

Dry resin was placed inside a round-bottom flask. Dry THF was added to swell the resin under nitrogen. 1M BH_3-THF (10 equiv. per amide bond) was slowly added at room temperature. When bubbles ceased, the flask was equipped with a condenser and gently

stirred at 65 °C for 24 h under nitrogen. Upon cooling to room temperature, the resin was transferred to a PP vessel and washed with dry THF. The resin was dried for 3 h and transferred to a round bottle flask following addition of piperidine 1 mL per 100 mg resin. After gently stirring at 65 °C for 16 h, the resin was washed with THF (3x), MeOH (3x) and DCM (3x), dried *in vacuo* 24 h.

2.5.2 Synthesis of starting materials

2.5.2.1 Synthesis of L-alaninol and Fmoc-L-alaninol (23)

L-alaninol was prepared in 74% yield according to the literature procedure.[31] Fmoc-L-alaninol (**23**) was prepared in 90% yield according to the literature procedure.[32] Both ^{1}H NMR data of compounds L-alaninol and Fmoc-alaninol were consistent with the literature data.

L-alaninol:

TLC: R_f = 0.28 (*n*-BuOH : H_2O : HOAc = 4 : 1 : 1), stain by ninhydrin

^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm): 1.02 (d, 6.5 Hz, 3H), 2.32 (br. s, 3H), 2.95-3.02 (m, 1H), 3.20 (dd, 7.8, 10.6 Hz, 1H), 3.49 (dd, 4.0, 10.6 Hz, 1H).

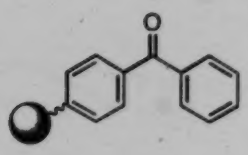
Fmoc-L-alanine:

TLC: R_f = 0.16 (hex : EtOAc = 1: 1)

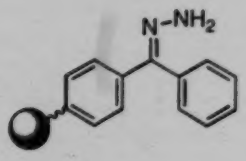
LC-MS: reverse phase gradient elution method 1 (Section 2.5.1.2), Rt = 8.951 min, %purity > 98%, ES-MS: *m/z* $(M+Na)^+$ = 320.1.

^{1}H NMR: (300 MHz, $CDCl_3$) δ(ppm): 1.14 (d, 6.2 Hz, 3H), 2.11 (br. s, 1H), 3.50 (br. s, 1H), 3.61 (br. s, 1H), 3.80 (br. s, 1H), 4.19 (t, 6.6 Hz, 1H), 4.41 (d, 6.5 Hz, 2H), 4.87 (br. s, 1H), 7.29 (t, 7.4 Hz, 2H), 7.38 (t, 7.4 Hz, 2H), 7.57 (d, 7.4 Hz, 2H), 7.74 (d, 7.4 Hz, 2H).

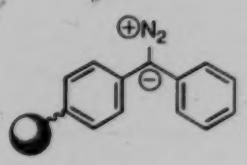
2.5.2.2 Synthesis of diphenyldiazomethane resin 39



37



38



39

Benzophenone resin **37** was prepared according to literature procedure.[48] To a round bottom flask equipped with a stir bar, was added 523 mg $AlCl_3$ (3.9 mmol), 3 mL dry DCM under nitrogen, followed by cooling to 0 °C. 0.45 mL benzoyl chloride (3.9 mmol) was then added to the above solution. This solution was added dropwise to 0.98 g polystyrene divinylbenzene resin which was pre-swelled in 15 mL DCM and cooled to –10 °C. The reaction was maintained at –10 °C for 30 min and room temperature for 3.5 h. Benzophenone resin **37** thus obtained was transferred to a PP vessel and washed with DCM, THF, 0.1N H_2SO_4 aqueous solution, MeOH and DCM three times each. Resin **37** was dried over high vacuum 24 h. The infrared spectrum showed the presence of carbonyl group with strong and sharp absorption at 1659 cm^{-1}.

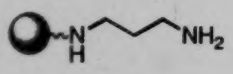
Benzophenone hydrazone resin **38** was prepared according to the literature procedure.[49] Resin **37** was suspended in 15 mL *n*-BuOH in a round bottom flask which was equipped with a condenser, followed by addition of 2 mL NH_2NH_2 (10 equiv.). The suspension was gently stirred and heated at 120 °C 16 h. The reaction mixture was cooled to room temperature and transferred to a PP vessel. The resin washed with THF, MeOH, and DCM three times each providing benzophenone hydrazone resin **38**. The infrared spectrum showed NH_2 bond absorptions at 3411 and 3328 cm^{-1}.

The procedure of synthesis of diphenyldiazomethane in solution phase[47, 50] was used for the synthesis of diphenyldiazomethane resin **39**.

0.12 g resin **38** was suspended in 7 mL DCM, followed by addition of catalytic I_2 (0.1 mL 0.1% I_2 in DCM) and 108 µl 1,1,3,3-tetramethylguanidine (3.5 equiv. respective to resin) under nitrogen. The mixture was cooled to –10 °C. 56 mg *m*-CPBA (1.3 equiv. respective to the resin) in 1.5 mL DCM was added dropwise to the resin suspension over

5 min and the mixture was maintained at –10 °C for 40 min and 10 min at room temperature. Under positive pressure of nitrogen, the violet color resin **39** was washed with THF x1 and DCM x4, dried over high vacuum for 16 h. Diphenyldiazomethane resin **39** was used for loading Fmoc-alaninol directly without analysis.

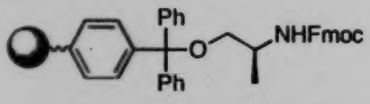
2.5.2.3 Synthesis of resin bound 1,3-propanediamine 62a



To 17.1 mL of 1,3-propanediamine (100 equiv. respective to resin) in a PP vessel, was added 1 g trityl chloride resin (2.05 mmol/g) in portion over 1 h at room temperature while shaking. The suspension was shaken for 3 h at room temperature followed draining and washing with DCM and MeOH. The resultant resin was suspended in MeOH and shaken 20 min at room temperature followed by washing with THF, MeOH and DCM three times each, and dried *in vacuo*.

2.5.3 Loading Fmoc-alaninol to resin

2.5.3.1 Loading Fmoc-alaninol to trityl chloride resin



Fmoc-alaninol was immobilized to trityl chloride resin according to the literature procedure.[33]

To a dry PP vessel charged with 100 mg trityl chloride resin (2.05 mmol/g) under nitrogen, was added 243 mg Fmoc-alaninol (0.82 mmol) followed by addition of pyridine 66.3 μl (0.82 mmol) in 5 mL THF in one portion. The PP vessel was capped, sealed and vortexed at room temperature for 4 days. The solution in the PP vessel was the filtered and collected, and the resin was washed with THF, MeOH and DCM three times each.

The wash solution was collected for recovery of Fmoc-alaninol. The resin was dried under high vacuum 16 h. Loading efficiency was 75%, which was measured by UV (Section 2.5.1.1) or elemental analysis of nitrogen.

To recover Fmoc-alaninol, the wash solution was collected. The combined organic solution was evaporated and re-dissolved in EtOAc followed by washing with water, brine and drying over Na_2SO_4. Removal of solvent provided Fmoc-alaninol.

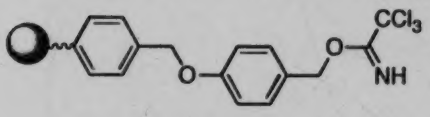
LC-MS: reverse phase gradient elution method 1 (Section 2.5.1.2)

Fmoc-alaninol: Rt = 8.978 min, ES-MS: *m/z* $(M+Na)^+$ = 320.1

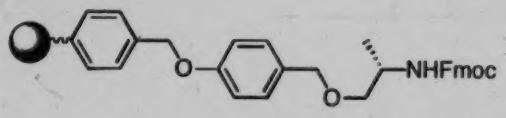
Fmoc-alaninol TFA ester: Rt = 9.663 min, ES-MS: *m/z* $(M+Na)^+$ = 416.1.

%purity (Fmoc-alaninol + Fmoc-alaninol TFA ester) > 98%.

2.5.3.2 Loading Fmoc-alaninol to Wang resin



26



27

Fmoc-alaninol was immobilized to Wang resin according to the literature procedure.[41]

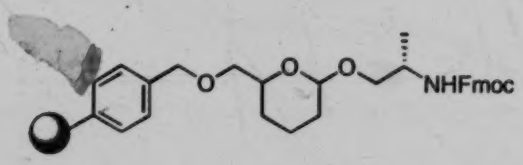
To a suspension of 1.1 g Wang resin (1.30 mmol/g) in dry DCM (10 mL) and THF (5 mL), was added 2.7 mL tricloroacetonitrile (18 equiv.). The mixture was cooled to 0 °C. 0.19 mL DBU (0.9 equiv.) was added dropwise to the suspension over 5 min, and then the solution was maintained at 0 °C for 40 min. The thus obtained brown resin **26** was washed with DCM, DMSO, THF and DCM three times each, dried *in vacuo*. The infrared spectrum shown the presence C=N bond at 1664 cm^{-1} and =N-H bond at 3339 cm^{-1}, the disappearance of O-H broad absorbance of original resin at 3150~3650 cm^{-1}.

0.17 g resin **26** was suspended in 4 mL dry DCM under a nitrogen atmosphere, followed by the addition of 130 mg Fmoc-alaninol (2 equiv.). The suspension was vortexed for 2 min. 9.4 μL $BF_3 \cdot Et_2O$ was added to this suspension and vortexed 5 min. The obtained resin **27** was washed with THF, MeOH and DCM three times each, dried *in vacuo* overnight. Loading efficiency was 82%, as measured by UV.

Release of compounds from Wang resin was achieved by treatment of resin with 5% TFA in DCM.

LC-MS: %purity > 95%

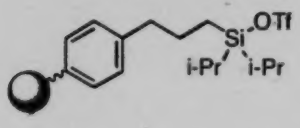
2.5.3.3 Loading Fmoc-alaninol to DHP resin



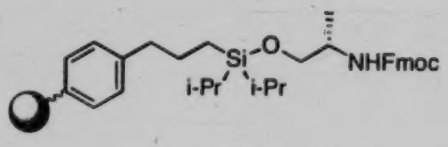
Fmoc-alaninol was immobilized to DHP resin according to the literature procedure.[45, 46] To the suspension of 0.31 g DHP resin (0.83 mmol/g) in a round-bottom flask equipped with a condenser, was added the solution containing 230 mg Fmoc-alaninol (0.78 mmol) and 98 mg PPTS (0.39 mmol). The suspension was heated at 80 °C for 16 h. The obtained resin **35** was washed with THF, MeOH and DCM three times each, dried *in vacuo*. Loading efficiency was 91%, as measured by UV. Release of compound from DHP resin was achieved by treatment of resin with 95% TFA/H_2O (95:5) for 20 min.

LC-MS: %purity > 95%

2.5.3.4 Loading Fmoc-alaninol to silyl resin



44



45

Fmoc-alaninol was immobilized to silyl resin according to literature procedure.[55, 56]

0.33 g silyl resin ((4-methoxyphenyl) diisopropylsilylpropyl polystyrene) (1.4 mmol/g) was suspended with dry DCM in a PP vessel under a nitrogen atmosphere. To this suspension, was added dropwise 245 μL TfOH (2.77 mmol) in 6 mL DCM under nitrogen. The suspension was allowed to vortex for 1.5 h at room temperature. The obtained dark red resin **44** was drained and washed with dry DCM three times under a positive nitrogen pressure. Resin **44** was used for loading Fmoc-alaninol immediately.

Resin **44** from above was suspended with dry DCM in a PP vessel under nitrogen atmosphere. To this suspension, was added 430 μL 2, 6-lutitine (3.7 mmol) at room temperature, followed by subjection to vortexing for 10 min. 270 mg Fmoc-alaninol (0.92 mmol) was added to above suspension and vortexed overnight under nitrogen. The resulting resin **45** was washed with DCM, THF, DMF, MeOH, THF and DCM three times each.

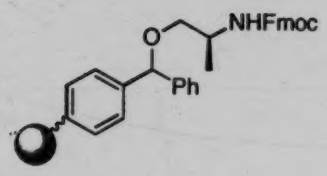
Cleavage of compounds from silyl resin for LC-MS:

To the suspension of a small amount (~1 mg) of resin **45** in a vial, was added 0.5 mL THF/HF/pyridine (95 : 3.5 : 1.5), which was obtained by diluting 0.5 mL HF/pyridine (7:3 from Aldrich Chemical Co.) with 9.5 mL dry THF. The suspension was sealed , followed by stirring at room temperature for 3 h. Unreacted HF was quenched by adding 25 μL methoxytrimethylsilane and was allowed to stir for additional 30 min. The suspension was filtered through a cotton plug in a glass pipette and rinsed with 0.5 mL MeOH. After evaporation of combined organic solution, the residue dissolved in 0.5 mL MeOH (HPLC grade), then filtered through a 0.2 μm filter tip.

Loading efficiency was 91%, which was measured by UV.

LC-MS: %purity > 95%

2.5.3.5 Loading Fmoc-alaninol to diphenyldiazomethane resin



40

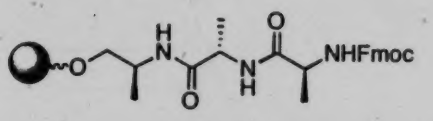
Fmoc-alaninol was immobilized to diphenyldiazomethane resin according to the literature procedure.[47]

0.12 g diphenyldiazomethane resin **39** was suspended in 7 mL DCM followed addition of 148 mg Fmoc-alaninol (0.50 mmol), then vortexed for 2 min. Catalytic $BF_3 \cdot Et_2O$ (3 µl, 0.025 mmol) was added to the mixture under nitrogen following vortexing for 4 h at room temperature. The obtained resin **40** was washed with THF, DCM and MeOH three times each and dried over high vacuum for 16 h.

LC-MS: %purity > 95%.

2.5.4 Synthesis of trityl resin bound Dde protected triamine 24

2.5.4.1 Synthesis of resin bound tripeptide 17a



Resin bound tripeptide **17a** was prepared from resin bound Fmoc-alaninol **16** according to the standard Fmoc-amino acid coupling procedure (Section 2.5.1.3).

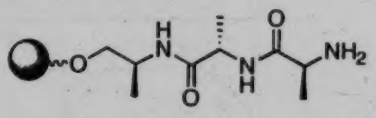
LC-MS: reverse phase gradient elution method 1 (Section 2.5.1.2)

Fmoc-alaninol: Rt = 12.281 min, ES-MS: *m/z* $(M+H)^+$ = 440.2

Fmoc-alaninol TFA ester: Rt = 13.794 min, ES-MS: *m/z* $(M+H)^+$ = 536.2

%purity (Fmoc-alaninol + Fmoc-alaninol TFA ester) > 98%.

2.5.4.2 Synthesis of resin bound tripeptide 17

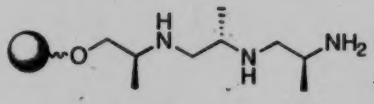


Resin bound tripeptide **17** was prepared by treatment of resin **17a** with 20% piperidine/DMF.

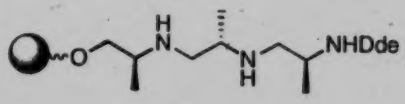
LC-MS: reverse phase gradient elution method 1 (Section 2.5.1.2), Rt = 1.400 min, ES-MS: *m/z* $(M+H)^+$ = 218.1.

^{1}H NMR: (300 MHz, CD_3OD) δ(ppm): 1.13 (d, 6.6 Hz, 3H), 1.36 (d, 7.2 Hz, 3H), 1.50 (d, 7.2 Hz, 3H), 3.43-3.48(m, 2H), 3.83-3.97 (m, 2H), 4.35 (q, 7.1 Hz, 1H).

2.5.4.3 Synthesis of resin bound triamine 18 and 24



18



24

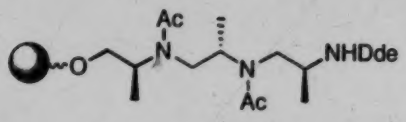
Resin **18** was prepared from resin **48** according to the general reduction procedure (Section 2.5.1.5). ES-MS: *m/z* $(M+H)^+$ = 190.2

To the suspension of resin **18** in DMF in a PP vessel, was added 1.1 equiv. Dde-OH and 4 equiv. Et_3N followed by shaking at room temperature for 3 h. The resulting orange color resin was washed with DMF, THF, MeOH and DCM three times each, then dried over high vacuum for 16 h. The standard cleavage procedure provided Dde protected triamine alcohol **24**.

LC-MS: reverse phase gradient elution method 1 (Section 2.5.1.2), Rt = 5.247 min, ES-MS: *m/z* $(M+H)^+$ = 354.2.

% purity > 95%

2.5.4.4 Synthesis of resin bound Dde protected triamine 24



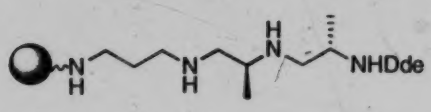
1.0 g of resin **18** was suspended in 10 mL DMF in a PP vessel followed by addition of 0.4 mL Et_3N and 1 mL Ac_2O. The mixture was vortexed at room temperature for 3 h, and then drained, washed with DMF, THF, MeOH and DCM three times each, and dried over high vacuum for 16 h.

LC-MS: reverse phase gradient elution method 3 (Section 2.5.1.2),

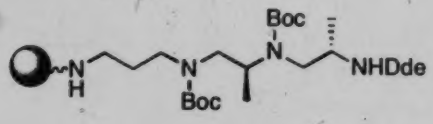
Rt = 8.424 min, ES-MS: *m/z* $(M+H)^+ = 438.2$.

% purity > 95%

2.5.5 Synthesis of Boc protected polyamines 65



62



65

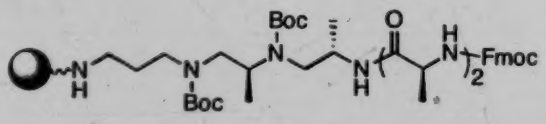
Resin **62** was prepared from **62a** using the same procedures as for the synthesis of **24**.

To the suspension of resin **62** in DCM in a PP vessel, was added 6 equivalents of di-*tert*-butyl dicarbonate and 6 equivalents of DIPEA in DCM. The suspension was vortexed at room temperature 40 h followed by washing with THF, MeOH and DCM three times each to provide resin **65**.

To evaluate this step, a small amount (~1 mg) of resin **65** was placed inside of a PP vessel followed wash with THF and DMF twice each, then suspended in DMF. To this suspension, was added 20 μL Et_3N and 50 μL acetic anhydride followed by vortexing for 3 h at room temperature. The resulting resin was washed with THF, MeOH and DCM, followed by treatment with 30% TFA/DCM to release compound from the resin for LC-MS analysis. No acetylated compounds were found.

LC-MS: reverse phase gradient elution method 1 (Section 2.5.1.2), Rt = 7.884 min. *m/z* $(M+H)^+$ = 353.3 (Boc groups were removed under this cleavage condition).

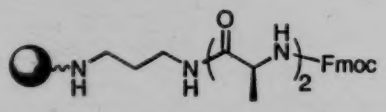
2.5.6 Synthesis of peptide 66



Resin **65** in a PP vessel was treated with 2% NH_2NH_2/DMF for 30 min at room temperature twice. LC-MS showed Dde group was removed. The resulting resin was coupled with Fmoc-alanine twice to generate resin **66** by using standard Fmoc AA coupling methods (Section 2.5.1.3). The resulting resin was washed with THF, MeOH and DCM, followed by treatment with 30% TFA/DCM to release the compounds from the resin for LC-MS analysis.

LC-MS of **66**: reverse phase gradient elution method 1 (Section 2.5.1.2), Rt = 10.348 min. ES/MS: *m/z* $(M+H)^+$ = 553.4 (Boc groups were removed under this cleavage condition). % purity > 90%.

2.5.7 Synthesis of resin bound peptide 75

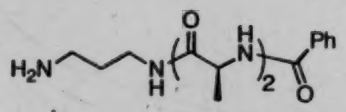


Resin bound peptide **75** was prepared from resin **62a** according to the general procedure for peptide synthesis (Section 2.5.1.3). **75** gave negative Kaiser test.

LC-MS: reverse phase gradient elution method 3 (Section 2.5.1.2), Rt = 9.790 min, ES-MS: *m/z* $(M+H)^+$ = 439.2.

% purity > 98%

2.5.8 Synthesis of peptide 77



Resin **75** in a PP vessel was treated with 20% piperidine/DMF for 30 min. at room temperature followed by washing with DMF, THF, MeOH and DCM three times each. The resultant resin was suspended in THF. To this suspension, was added 8 equiv. DIPEA and 4 equiv. benzoyl chloride in THF followed by shaking for 3 h at room temperature. The standard cleavage procedure (Section 2.5.1.4) provided **77**.

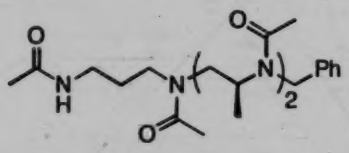
LC-MS: reverse phase gradient elution method 3 (Section 2.5.1.2),

Rt = 6.960 min, ES-MS: *m/z* $(M+H)^+$ = 321.2, % purity > 98%

^{1}H NMR: (300 MHz, CD_3OD) δ(ppm): 1.37 (d, 7.2 Hz, 3H), 1.48 (d, 7.2 Hz, 3H), 1.85 (quint, 6.8 Hz, 2H), 2.96 (t, 7.1 Hz, 2H), 3.30 (2H overlap with CD_3OD), 4.24 (q, 7.2 Hz, 1H), 4.43 (q, 7.2 Hz, 1H), 7.43-7.51 (m, 2H), 7.52-7.59 (m, 1H), 7.85-7.92 (m, 2H).

^{13}C NMR: (125 MHz, CD_3OD) δ(ppm): 17.4 (CH_3), 17.5 (CH_3), 28.6 (CH_2), 36.9 (CH_2), 38.1 (CH_2), 51.1 (CH), 51.9 (CH), 128.6 (CH), 129.5 (CH), 133.0 (CH), 134.9 (C), 170.6 (CO), 175.6 (CO), 175.9 (CO).

2.5.8.1 Synthesis of acetylated polyamine 79



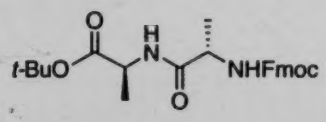
0.37 g compound **77** dissolved in dry THF in a round-bottom flask equipped with a condenser, magnetic stir bar and nitrogen inlet. The solution was cooled to 0 °C, followed by dropwise addition of 23 mL 1M BH_3-THF (30 equiv.) over 10 min under nitrogen. Once bubbles ceased, the solution was heated at 65 °C for 2 days under nitrogen. When the reaction was complete, the reaction solution was cooled to room temperature and

further to 0 °C. 5 mL dry MeOH was added dropwise and carefully. Once bubbles ceased, the solution was heated at reflux overnight. Evaporation of solvent provided free polyamine **78** (ES-MS: *m/z* $(M+H)^+$ = 279.2).

The solution of crude polyamine **78** in 3 mL THF was cooled to 0 °C followed by addition of 850 µL Et_3N (12 equiv.) and 190 µL acetic anhydride (6 equiv.). The reaction solution was stirred overnight at room temperature. Evaporation of the solvent provided the crude compound **79** in 75% purity (HPLC-LC-MS).

LC-MS: reverse phase gradient elution method 3 (Section 2.5.1.2), Rt = 8.495 min, ES-MS: *m/z* $(M+H)^+$ = 447.3, % purity: 75%.

2.5.9 Synthesis of dipeptide 90



Dipeptide **90** was prepared according to the literature procedure.[3]

181 mg L-alanine tert-butyl ester hydrochloride (1.0 mmol), 311 mg Fmoc-L-alanine (1.0 mmol), 153 mg 1-hydroxy benzotriazole monohydrate (1.0 mmol), and 127 µL N-ethylmorpholine (1.0 mmol) were dissolved in 3 mL dry THF. The solution was cooled to 0 °C and stirred. To this solution, was added 216 mg DCC (1.05 mmol) at 0 °C and the solution was maintained 1 h then at room temperature 1 h. The formed white solid was filtered and washed with THF. The combined THF solution was evaporated. The residue was re-dissolved in 10 mL EtOAc followed by sequential washing with saturated aqueous $NaHCO_3$, 10% aqueous citric acid, saturated aqueous $NaHCO_3$, water and brine. The solution was dried over Na_2SO_4. Evaporation of solvent generated crude compound. Dipeptide **90** (427mg, 97%) was purified by flash silica gel chromatography (Hex : EtOAc = 2 :1 to 1:1).

TLC: R_f = 0.38 (Hex : EtOAc = 1 : 1)

mp: 156.6-157.9 °C.

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.34 (d, 7.1 Hz, 3H), 1.38 (d, 6.2 Hz, 3H), 1.44 (s, 9H), 4.02 (t, 7.1 Hz, 1H), 4.24 (br. 1H), 4.33-4.46 (m, 3H), 5.40 (br. 1H), 6.40 (br. 1H), 7.26 (t, 7.4 Hz, 2H), 7.34 (t, 7.4 Hz, 2H), 7.54 (br. d, 6.6 Hz, 2H), 7.71 (d, 7.6 Hz, 2H).

^{13}C NMR (500 MHz, $CDCl_3$) δ(ppm): 18.5 (CH_3), 19.0 (CH_3), 27.9 (CH_3), 47.1 (CH), 48.7 (CH), 50.4 (CH), 67.1 (CH_2), 82.1 (C), 119.9 (CH), 125.1 (CH), 127.1 (CH), 127.7 (CH), 141.3 (C), 143.80 (C), 143.85 (CO), 171.6 (CO), 171.8 (CO).

HPLC: reverse phase gradient elution method 3 (Section 2.5.1.2), Rt: 12.499 min.

HR-MS for molecular formula $C_{25}H_{30}N_2O_5$: *m/z* $(M+Na)^+$ calcd. 461.20469, found: 461.20430.

2.5.10 Synthesis of tripeptide Fmoc-(Ala)$_3$-O*t*-Bu (93)

Fmoc-Ala-Ala-OH (**91**) was prepared from dipeptide **90** by removal of the *t*-Bu group. 194 mg Fmoc-Ala-Ala-O*t*-Bu (**90**) (0.44 mmol) was dissolved in 2.2 mL 1M HCl/HOAc followed by stirring at room temperature. The reaction, monitored by TLC, was complete after 8 h. Solvents were evaporated by rotary evaporation and oil pump, dried *in vacuo* to provide Fmoc-Ala-Ala-OH (**91**) 168 mg (99% yield) as a white solid.

Tripeptide Fmoc-(Ala)$_3$-O*t*-Bu (**93**) was prepared by coupling of **91** with Fmoc-L-alanine in 95% yield using the same procedure of the synthesis of Fmoc-Ala-Ala-O*t*-Bu (**90**) (Section 2.5.9).

Fmoc-Ala-Ala-OH (**91**)

TLC: R_f = 0.60 (DCM : MeOH : HOAc = 76 : 16 : 7.5)

^{1}H NMR (300 MHz, CD_3OD) δ(ppm): 1.33 (d, 1.7 Hz, 3H), 1.38 (d, 7.2 Hz, 3H), 4.01-4.26 (m, 2H), 4.30-4.43 (m, 3H), 7.29 (td, 7.5, 1.2 Hz, 2H), 7.37 (t, 7.2 Hz, 2H), 7.65 (t, 7.1 Hz, 2H), 7.77 (7.5 Hz, 2H).

Fmoc-(Ala)$_3$-O*t*-Bu (**93**)

TLC: R_f = 0.35 (DCM : MeOH : HOAc = 95 : 5 : 1)

mp: 164.2-165.8 °C.

^{1}H NMR (500 MHz, $CDCl_3$) δ(ppm): 1.34 (d, 6.8 Hz, 3H), 1.39 (d, 6.9 Hz, 6H), 1.44 (s, 9H), 4.20 (t, 7.0 Hz, 1H), 4.30-4.47 (m, 4H), 4.57 (quint, 7.0 Hz, 1H), 5.70 (br, 1H), 6.86 (br, 1H), 6.94 (br, 1H), 7.29 (t, 7.4 Hz, 2H), 7.38 (t, 7.5 Hz, 2H), 7.58 (d, 7.4 Hz, 2H), 7.75 (d, 7.5 Hz, 2H).

^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm): 18.4 (CH_3), 18.7 (CH_3), 19.0 (CH_3), 27.9 (CH_3), 47.1 (CH), 48.76 (CH), 48.86 (CH), 50.5 (CH), 67.1 (CH_2), 82.0 (C), 119.9 (CH), 125.0 (CH), 127.0 (CH), 127.7 (CH), 141.3 (C), 143.8 (C), 155.9 (CO), 171.4 (CO), 171.8 (CO), 172.1 (CO).

LC-MS: reverse phase gradient elution method 2 (Section 2.5.1.2), Rt: 11.926 min, *m/z* $(M+H)^+$ = 510.2.

HR-MS for molecular formula $C_{28}H_{35}N_3O_6$: *m/z* $(M+Na)^+$ calcd. 532.24181 found: 532.24151.

2.5.11 Synthesis of Fmoc-$(Ala)_4$-O*t*-Bu (95)

Fmoc-$(Ala)_3$-OH (**94**) was prepared in quantitative yield from tripeptide **93** using the same procedure of the synthesis of Fmoc-$(Ala)_2$-OH (**91**).

To the solution of Fmoc-$(Ala)_3$-OH (**94**) (0.938 g, 2.0 mmol) in 9 mL dry DMF, was added 260 μL N-ethylmorpholine (2.0 mmol). The solution was cooled to –15 °C followed by addition of 200 μL ethyl chlorocarbonate (2.0 mmol) under nitrogen atmosphere. The resulting solution was stirred in a minute at –15 °C, followed by addition pre-cooled 4 mL DMF solution (–15 °C) containing 376 mg L-alanine-*tert*-butyl ester hydrochloride (2.0 mmol) and 260 μL *N*-ethylmorpholine (2.0 mmol). The solution was stirred overnight at room temperature. After the solvent was evaporated, the resultant residue was re-dissolved in 10 mL DMF. The slow addition of this solution to 100 mL water during stirring, produced a white precipitate that was filtered and washed with water three times, dried *in vacuo* to provide Fmoc-$(Ala)_4$-O*t*-Bu (**95**) 0.975g (81% yield).

Fmoc-$(Ala)_3$-OH (**94**)

TLC: R_f = 0.25 (DCM : MeOH : HOAc = 95 : 5 : 1)

^{1}H NMR (300 MHz, CD_3OD) δ(ppm): 1.30-1.42 (m, 9H), 4.12 (q, 7.2 Hz, 1H), 4.21 (t, 6.7 Hz, 1H), 4.28-4.42 (m, 4H), 7.30 (t, 7.3 Hz, 2H), 7.38 (t, 7.3 Hz, 2H), 7.65 (t, 7.2 Hz, 2H), 7.78 (d, 7.4 Hz, 2H).

Fmoc-$(Ala)_4$-O*t*-Bu (**95**)

TLC: R_f = 0.23 (DCM : MeOH : HOAc = 95 : 5 : 1)

mp: 167.5-168.8 °C.

^{1}H NMR (400 MHz, DMSO-d_6) δ(ppm): 1.16-1.24 (m, 12H), 1.36 (s, 9H), 4.05 (app. sept, 2H), 4.15-4.30 (m, 5H), 7.31 (t, 7.4 Hz, 2H), 7.40 (t, 7.4 Hz, 2H), 7.51 (d, 7.4 Hz, 1H), 7.7 (t, 6.9 Hz, 2H), 7.84 (d, 8.0 Hz, 1H), 7.87 (d, 7.5 Hz, 2H), 7.95 (d, 7.0 Hz, 1H), 8.12 (d, 6.8 Hz, 1H)

^{13}C NMR (100 MHz, DMSO-d_6) δ(ppm): 16.8 (CH_3), 18.1 (CH_3), 18.1 (CH_3), 18.3 (CH_3), 27.5 (CH_3), 46.6 (CH), 47.5 (CH), 47.9 (CH), 48.2 (CH), 49.9 (CH), 65.6 (CH_2), 80.2 (C), 120.1 (CH), 125.2 (CH), 127.0 (CH), 127.6 (CH), 140.7 (C), 143.7 (C), 143.8 (C), 155.6 (CO), 171.5 (CO), 171.7 (CO), 172.2 (CO), 172.2 (CO).

LC-MS: reverse phase gradient elution method 2 (Section 2.5.1.2). Rt = 12.142 min, *m/z* (M+Na)$^+$ = 603.2.

HR-MS for molecule formula $C_{31}H_{40}N_4O_7$: *m/z* (M+H)$^+$ calcd. 581.29698, found: 581.29703.

2.5.12 Synthesis of Synthesis of octapeptide 106

NH_2-$(Ala)_4$-O*t*-Bu (**104**)was prepared from tetrapeptide **95** according to the literature procedure.[3]

0.562 g tetrapeptide **95** (0.97 mmol) and 1.0 mL diethylamine were added to 8 mL DMF. The solution was stirred at room temperature 2 h. DMF was evaporated under reduced pressure. The resulting residue was triturated with Et_2O (3x5 mL), which was monitored by TLC, to provide white solid NH_2-$(Ala)_4$-O*t*-Bu (**104**) (0.270 g, 78% yield). Fmoc-$(Ala)_4$-OH (**105**) was prepared in quantitative from tetrapeptide **95** using the same procedure of the synthesis of Fmoc-$(Ala)_2$-OH (**91**) (TLC: R_f = 0.23 (DCM : MeOH : HOAc = 95 : 5 : 1)).

Octapeptide Fmoc-$(Ala)_8$-O*t*-Bu (**106**) was prepared in 62% yield from Fmoc-$(Ala)_4$-OH (**105**)and NH_2-$(Ala)_4$-O*t*-Bu (**104**) using the same procedure of synthesis of tetrapeptide **95**.

NH_2-$(Ala)_4$-O*t*-Bu (**104**)

^1H NMR (300 MHz, CD_3OD) δ(ppm): 1.27 (d, 7.0 Hz, 3H), 1.34 (d, 7.2 Hz, 3H), 1.358 (d, 7.2 Hz, 3H), 1.362 (d, 7.2 Hz, 3H), 3.44 (q, 1H), 4.23 (q, 7.2 Hz, 1H), 4.29-4.40 (m, 2H).

Fmoc-$(Ala)_8$-O*t*-Bu:

HR-MS for molecular formula $C_{16}H_{30}N_4O_5$: *m/z* $(M+H)^+$ calcd. 359.22890, found: 359.22884.

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Chapter 3 The Synthesis of Chiral Diamines

3.1 Introduction

In 1848, Pasteur hand-sorted the crystals grown from solutions of sodium ammonium paratartrate (racemic tartaric acid) into two sets.[1] One set had hemihedral facets oriented to the right and the other had hemihedral facets oriented to the left. These two types of crystals produced in solution a quantitatively similar specific optical rotation, but qualitatively with opposite sign. Pasteur surmised by analogy with the crystal forms that the two-identical-component substances had different molecular shapes, which were the non-superposable mirror images. Since then, chiral, diastereomer, enantiomer, isomer and other relative terminology were introduced. As we all know, proteins, DNA, enzymes, and other biological substances are made of small chiral molecules - amino acids, carbohydrates. People have realized that in most cases in nature, only one enantiomer or diastereomer displays strong biological activities. The synthesis of optically pure enantiomers in the laboratory was long a chemists' dream. During early stages, chemists only prepared racemic compounds because all starting materials, reagents, catalysts were racemic at that time. For a long time, chemists have made efforts to develop methods for the synthesis of optically pure compounds, and this topic still constitutes an ongoing subject of research. According to Willams's book,[2] those methods are classified into three different strategies: 1) kinetic resolution; 2) chiral pool strategies; 3) asymmetric synthesis. Catalytic asymmetric synthesis using achiral substances have attracted significant attention over the last three decades. In a chiral environment, such as that provided by reactions mediated by catalytic metal species complexed with chiral ligands, achiral substrates undergo reaction to generate optically enriched compounds.

Recently, rhodium and palladium have been widely used as metal species for asymmetric catalysis. Copper, zinc, nickel, cobalt and iron have also been used. Metal species complex ligands by electronic interaction between ligands and the metal ion, e.g., ligands donating electrons to a vacant metal orbital.[3] The central metal will act as Lewis acids to activate reactants. Ligands play a key role to control the stereochemical outcome. Because of steric hindrance or steric repulsion, one face of a substrate is blocked so that

reagents attack preferentially from the other face to induce enantiofacial discrimination. Among those ligands, bidentate ligands, such as BINAP, and binaphthol, are widely employed. Nitrogen-containing ligands, such as amino alcohols, diamines, and phosphine-nitrogen containing ligands, are also widely used.[4] Many groups reported diamines and diamine derivatives (Figure 3.1) as chiral ligands for asymmetric reactions, for instance, diamines **111**, **112** and **113** were used for hydrogenation of esters and ketones by the Lemaire group[5] and the Noyori group. [6] Other examples include **114** for enantioselective palladium(II)-catalyzed aerobic alcohol oxidations;[7] **115** for catalytic asymmetric allylation of hydrazono esters;[8] **116**, **117** and **118** for enantio-selective additions of diethylzinc to benzaldehyde;[9, 10] **119** for asymmetric transfer hydrogenation of benzaldehydes;[11] **120** for ytterbium-catalyzed asymmetric Diels-Alder reactions.[12]

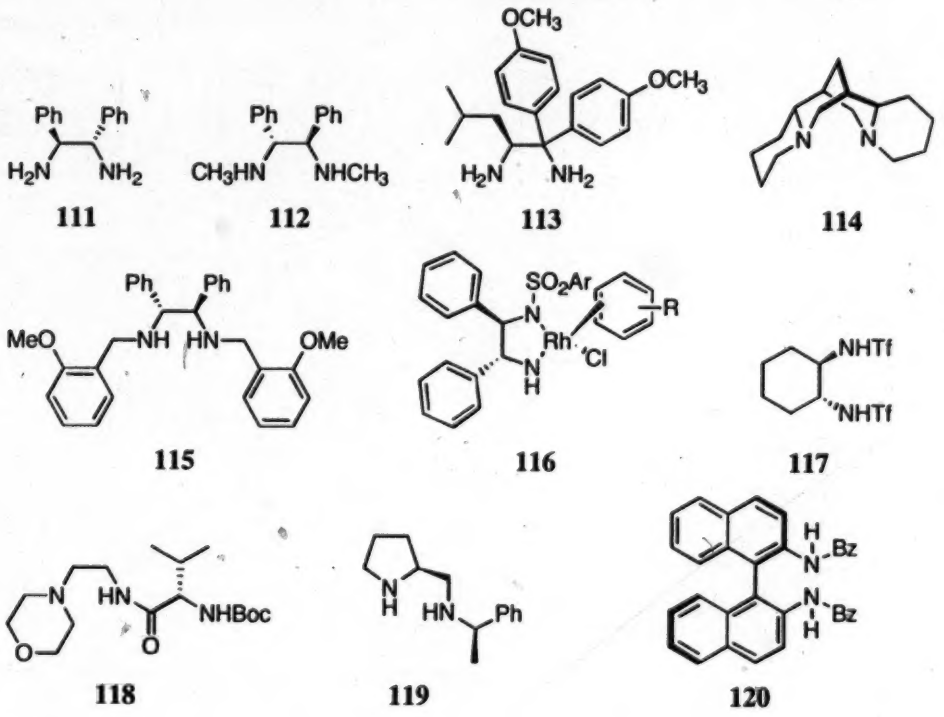
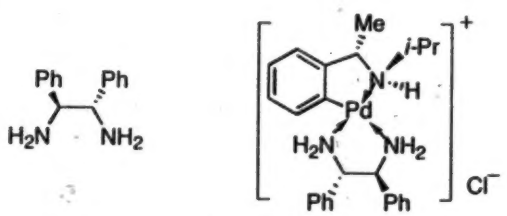


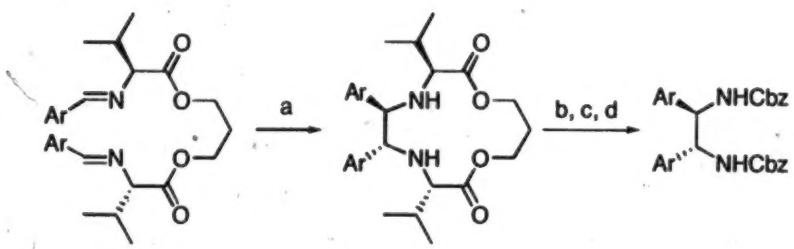
Figure 3.1 Chiral diamines and chiral diamine derivatives used as ligands

As mentioned above, three different strategies, which include kinetic resolution, chiral pool strategies and asymmetric synthesis, can be used for the syntheses of chiral diamines. For example, diamine **111** (Figure 3.1) and its derivatives, which are widely used in asymmetric reactions, were synthesized by these different strategies. The Corey group and the Dunina group reported kinetic resolution strategies to provide the diamine **111** by recrystallization of the diastereomers of L-(+)-tartaric acid salts and *ortho*-palladated complexes **121** (Figure 3.2) respectively.[13, 14] Starting from chiral material, the chiral diamines **122** (Scheme 3.1) were synthesized by the Shono group, and **123** (Scheme 3.2) was synthesized by the Burrows group.[15, 16] The Salvadori group and the Fujisawa group used chiral ligands to induce stereogenic centers in the synthesis of the diamines **124** (Scheme 3.3) and **125** (Scheme 3.4).[17, 18]



111 **121**

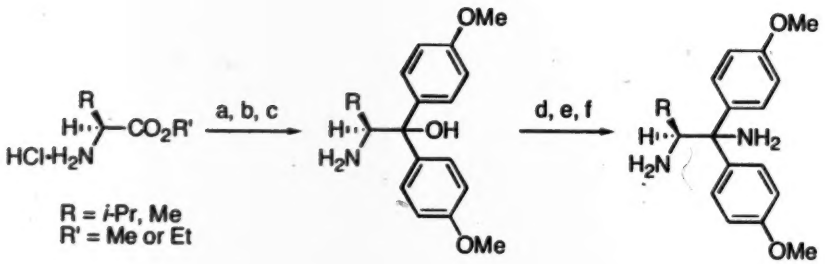
Figure 3.2 The structures of diamine **1** and its *ortho*-palladated complex **121**



122

Reaction conditions: (a) Zn, 0 °C, MsOH, THF. (b) NaOH/aq EtOH. (c) $Pb(OAc)_4/H_2O$. (d) CbzCl.

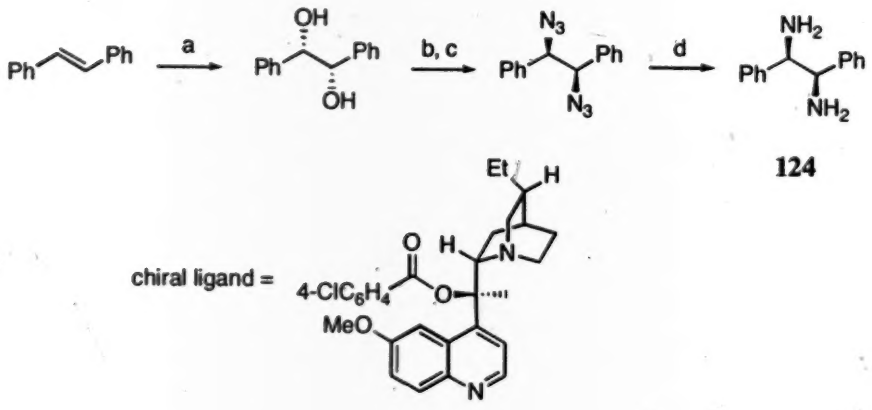
Scheme 3.1 The synthesis of chiral diamine **122** by the Shono group[15]



123

Reaction conditions: (a) *p*-$MeOC_6H_5MgBr$, THF. (b) HCl. (c) NH_4OH. (d) CbzCl, Pyridine. (e) NaN_3, TFA. (f) H_2, EtOH, 10% Pd/C

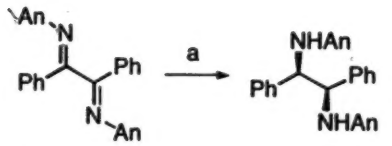
Scheme 3.2 The synthesis of chiral diamine **123** by the Burrows group[16]



124

Reaction conditions: (a) OsO_4, acetone, NMO, chiral ligand. (b) TsCl, Pyridine. (c) NaN_3, 90 °C, DMF. (d) $LiAlH_4$, Et_2O.

Scheme 3.3 The synthesis of chiral diamine **124** by the Salvadori group[17]



An = *p*-$MeOC_6H_4$ **125**

Reaction conditions: (a) BH_3/THF, chiral ligand.

Scheme 3.4 The synthesis of chiral diamine **125** by the Fujisawa group[18]

3.2 Perspective and objective of the project

Although a few groups already reported the use of different chiral diamines and chiral diamine derivatives as ligands in asymmetric reactions (Section 3.1), the current availability of chiral diamines is quite limited.

The first goal of this project is to develop a general method for the synthesis of new types of chiral diamines, which can provide an efficient high-throughput access to these important ligands from readily available amino acids. The second goal is to evaluate a number of representative diamines as chiral ligands for applications in asymmetric organic reactions, e. g., catalytic asymmetric allylation of hydrazono esters;[8] enantio-selective palladium (II)-catalyzed aerobic alcohol oxidations;[7] and asymmetric transfer hydrogenation of benzaldehydes.[11]

The easiest way to induce stereogenic centers in diamines is to take advantage of the "chiral pool," i. e., natural amino acids. As discussed in Chapter 2, solid-phase methods are particularly efficient to build polyamines by using our group's previously optimized protocol (Chapter 2, Scheme 2.12). To anchor a precursor to resin, an amine is required. To neutralize this amine, a suitable capping group is needed after cleavage from the resin. Considering several effects, the generic structure of our chiral diamine targets is **126** (Figure 3.3). The inert non-enolizable properties of the pivalic group make the resulting amide stable to nucleophiles and bases. The anchoring unit is designed with a

rigid piperidine ring structure, which will make metal coordination between the pivalic carbonyl and the internal amine conformationally unfavorable (Figure 3.3). Therefore, only the two terminal secondary or tertiary amine nitrogens can be involved as metal binding sites. When X, X = H, it will be a secondary diamine, whereas X, X = alkyl groups, it will be a tertiary diamine, which can be either acyclic or cyclic.

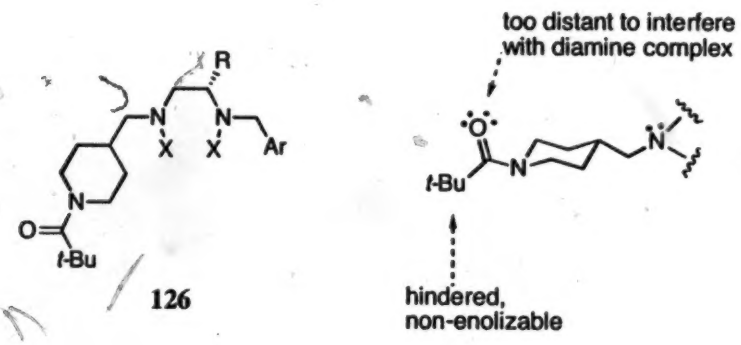
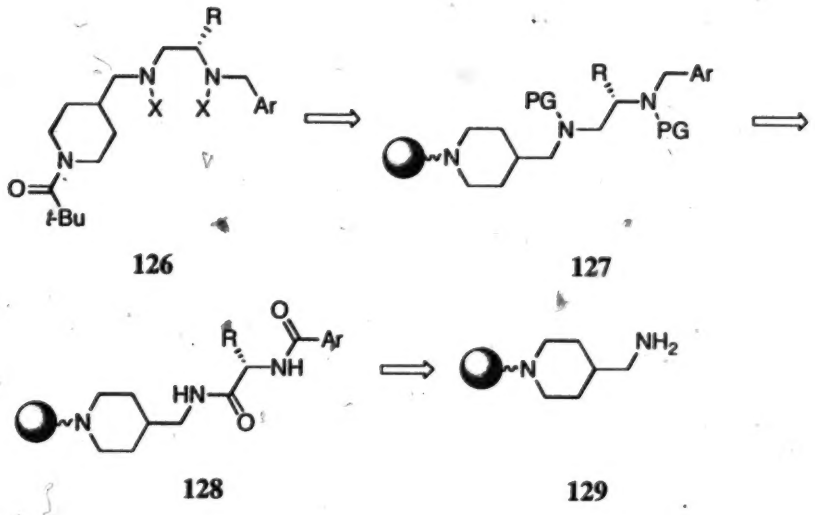


Figure 3.3 Generic structure of diamines

Chiral diamine **126** could be efficiently generated from resin bound diamine **127** via a short synthetic sequence consisting of protecting and deprotecting the amines, cleavage from the resin and finally capping the anchoring nitrogen (Scheme 3.5). Diamine **127** in turn could be obtained from exhaustive reduction of resin bound amide **128**, and the latter could be prepared from resin **129** using solid phase peptide synthesis methods.



126 **127**

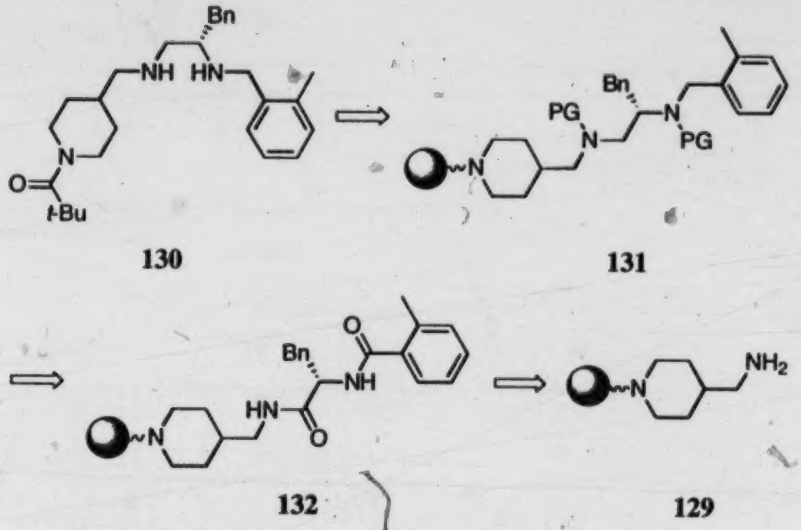
128 **129**

Scheme 3.5 Retrosynthetic approach to chiral diamines

3.3 Synthesis of chiral diamines

As mentioned in the introduction (Section 3.2), diamines are efficiently synthesized using solid-phase chemistry. The types of diamines that we were interested in are shown as a generic structure (Figure 3.3).

As discussed in Section 3.2, diamine **130** could be made through solid-phase chemistry. The retrosynthetic route is shown in Scheme 3.6. Diamine **130** could be generated from the resin bound protected diamine **131**, which can be obtained by reduction of resin bound peptide **132**.

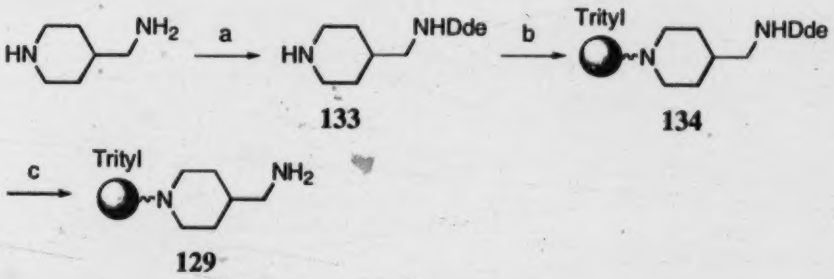


130 131 132 129

Scheme 3.6 Retrosynthetic approach for diamine **130**

3.3.1 Synthesis of resin bound piperidine linker 129 and diamine 135

Resin **129** was required to anchor the diamine precursors, but it was not commercially available. Thus, the preparation of resin **129** was carried out as shown in Scheme 3.7.



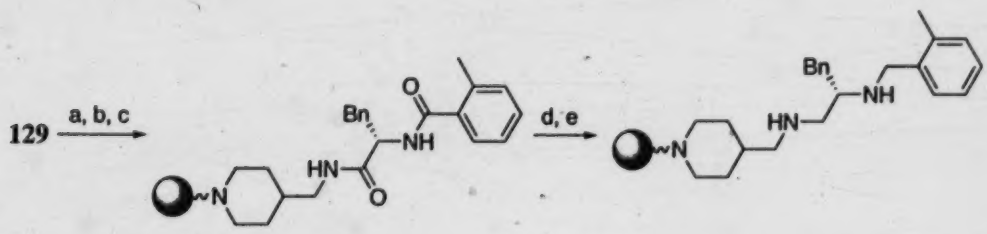
133 134 129

Reaction conditions: (a) 4-(aminomethyl)piperidine (1 equiv.), Dde-OH (1.05 equiv.), THF, rt, 1 h. (b) trityl chloride resin (1 equiv.), 4-(Dde-aminomethyl) piperidine (4 equiv.), DIPEA (4 equiv.), DCM, rt, 3 h. (c) 2% NH_2NH_2/DMF, rt, 30 min.

Scheme 3.7 Preparation of resin bound piperidine linker **129**

The linker 4-(Dde-aminomethyl)piperidine (**133**) was prepared by treatment of 4-(aminomethyl)piperidine with a slight excess of Dde-OH in THF. [19]After evaporating the solvent, the crude compound was obtained as a yellow solid in a quantitative yield and 96% purity (HPLC-LC-MS). The crude compound **133** was utilized for the next step without further purification. Resin **129** was achieved by loading 4-(Dde-aminomethyl) piperidine to trityl chloride resin in the presence of DIPEA. Any possible unreacted sites on the trityl resin were quenched with MeOH at the end of the reaction. LC-MS analysis showed a high purity of resin **129**. To remove the Dde protecting group, 2% NH_2NH_2 in DMF was applied. In this step, the presence of bulk blue beads in the Kaiser test did not necessarily mean that all Dde groups were removed. In our first trial, after removing the Dde group and coupling with Fmoc-phenylalanine, free 4-(Dde-aminomethyl)piperidine (**133**) was detected by LC-MS. This incomplete coupling could be rationalized by difficulty of the NH_2NH_2 solution to reach some pores inside of the resin. So, to probe the efficiency of the Dde group deprotection step, LC-MS analysis was more reliable than the Kaiser test. Increasing the reaction time and repeating the treatment with 2% NH_2NH_2 forced the deprotection step to completion.

From resin **129**, standard Fmoc-AA coupling provided resin bound peptide **132**, followed by reduction and work-up to give resin bound diamine **135**.



132 **135**

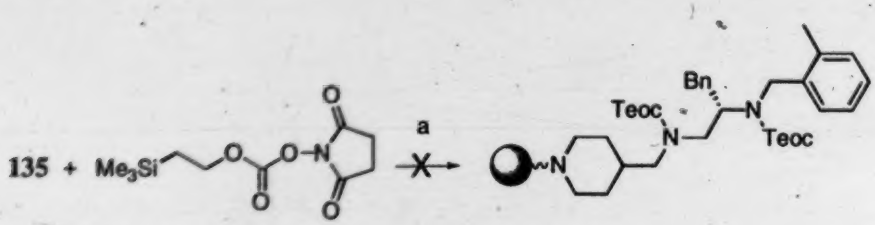
Reaction conditions: (a) HBTU, HOBt, DIPEA, Fmoc-L-phenylalanine. (b) 20% piperidine/DMF. (c) HBTU, HOBt, DIPEA, *o*-toluic acid. (d) BH_3/THF, 65 °C, 2 days. (e) piperidine, 65 °C,16 h.

Scheme 3.8 Preparation of resin bound diamine **135**

3.3.2 Synthesis of diamine 130

It was planned that following the cleavage step, the anchoring primary amine would be capped by a pivalic group. As such, the two free secondary amino nitrogen atoms in resin **135** must be protected before releasing the diamine from the resin. A suitable protecting group then must have these properties: 1) completely protect the two free secondary diamine nitrogens on resin **135**; 2) be tolerant to the subsequent cleavage conditions and the conditions for capping the anchoring amine by pivalic anhydride; 3) should be easily removed under mild conditions. In the solid-phase synthesis of exo-peptides described in this thesis, trityl, Nbs and Boc have been tested for protecting polyamines. Only the Boc group can afford fully protected polyamines, but it is not resistant to acidic conditions during cleavage.

The Rich group[20] reported 2-(trimethylsilyl)ethoxycarbonyl (Teoc) as a good protecting group for amino groups and using Teoc-OSu (1-[2-(trimethylsilyl) ethoxycarbonyloxy] pyrrolidin-2,5-dione) gave excellent yields without any evidence of impurity. The Teoc group is stable under basic and hydrogenolytic conditions, and its cleavage can be carried out using TBAF, strong acids, or Lewis acids. The Teoc group was successfully utilized in solid phase on DHP, chlorotrityl, and Wang resin.[21, 22, 23] Upon these literature results, we decided to test the Teoc group. When resin **135** was treated with Teoc-OSu, mono protected compounds were the major products as shown by two large peaks in the LC-MS (Scheme 3.9), along with small amounts of the desired diprotected compound. Trimethylsilyl groups may contribute to this incomplete protection because of steric hindrance.



136 137

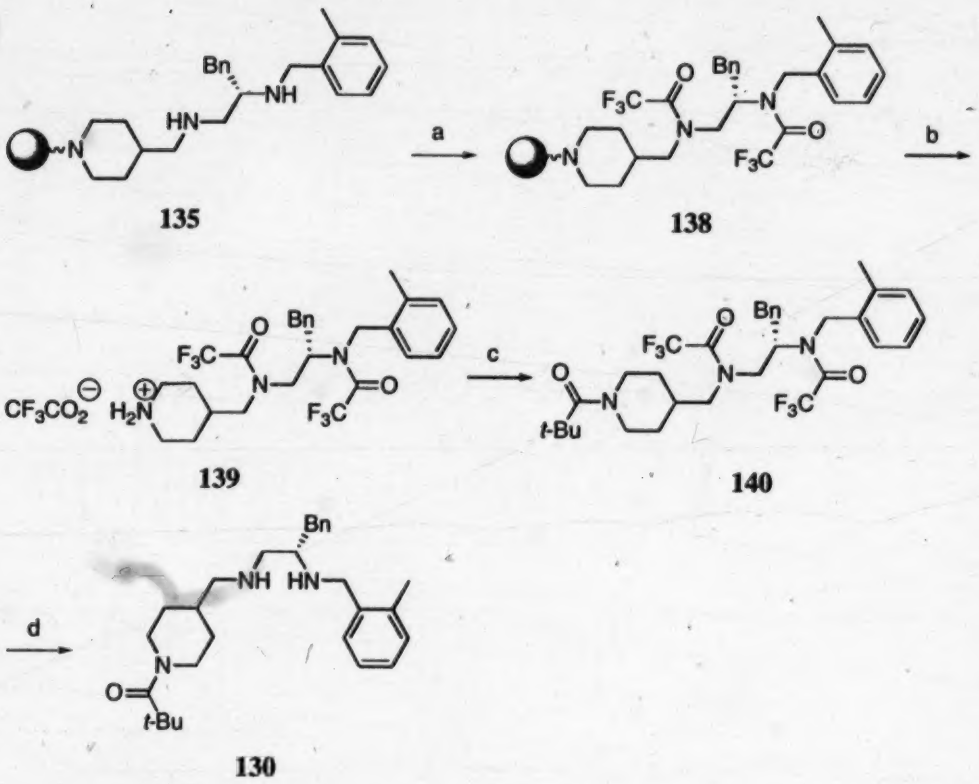
Reaction conditions: resin **135** (1 equiv.), Teoc-OSu (4 equiv.), Et_3N (8 equiv.), DMAP (1 equiv.), DCM, rt, 16 h.

Scheme 3.9 Protection of resin bound diamine **135** with Teoc group

Clearly, a smaller protecting group was required. Considering the requirements for protecting groups in this approach, an alternative could be the trifluoroacetyl group. It was not clear whether trifluoroacetyl group would afford full protection. It was thought to be possible because acetyl group, similar in size, and used in Section 2.3.1.1 (Chapter 2) provided the protected polyamine in 95% yield. Gratifyingly, resin **135** was fully protected and no mono-protected compound was observed in the LC-MS (Scheme 3.10).

Resin **138** was subjected to 5% TFA/DCM, releasing the compound from the polymer to give the TFA salt **139** as a slight yellow solid. It was pure by LC-MS, but its ^{1}H-NMR spectra was complicated due to the existence of the compound as a mixture of different tertiary amide isomers. This TFA salt **139** was used directly for next step without further purification.

The anchoring amine was then capped by acylation with pivalic anhydride providing compound **140**. Pre-purification by acid-base extraction in EtOAc with 1N HCl and 5% $NaHCO_3$ removed most of the polar impurities, such as triethylamine and its salt. Flash silica column chromatography generated the pure compound **140**, which showed one spot by TLC and high purity by LC-MS analysis. ^{1}H-NMR and ^{13}C-NMR were not suitable to evaluate its purity because the presence of tertiary amide rotamers. To verify its purity and structure, HR-MS and elemental analysis were performed. Both results were consistent with the calculated data for the expected product.



135 138 139 140 130

Reaction conditions: (a) resin **135** (1 equiv.), $(CF_3CO)_2O$ (10 equiv.), pyridine (10 equiv.), rt, 3 h. (b) 5% TFA/DCM, 30 min. (c) TFA salt **139** (1 equiv.), $(t\text{-BuCO})_2O$ (3 equiv.), Et_3N (3 equiv.), THF, rt, 6 h. (d) 7% K_2CO_3 in MeOH/H_2O (v/v 5:2), rt, 24 h.

Scheme 3.10 Preparation of diamine **130**

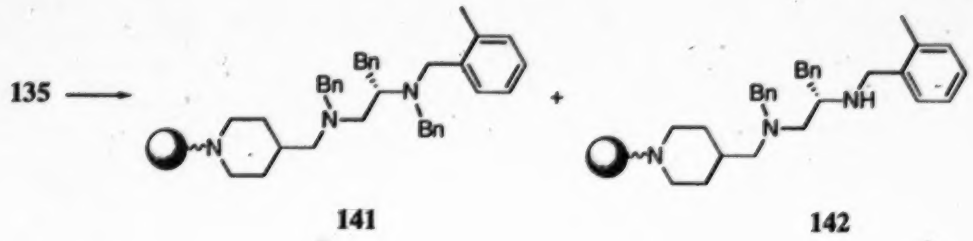
The last step to assemble the diamine **130** was to deprotect the trifluoroacetyl group. Several methods have been reported to remove trifluoroacetyl groups, including: 1) potassium carbonate in aqueous methanol at reflux or room temperature;[24, 25] 2) solid-liquid transfer strategy (H_2O/organic solvent with benzyltriethylammonium chloride and potassium carbonate) from 18 h to 7 days;[26] 3) Strongly basic anion exchange resin Lewatit 500.[27]

Compared to other systems, the protocol using potassium carbonate took the shortest amount of time and is benefited by the cheap price of the reagent. Both room temperature and refluxing conditions were investigated. The reactions were monitored by TLC. Under refluxing conditions, two large spots on TLC formed at the same time. One spot represented the desired compound (m/z $(M+H)^+$ = 436.3) and the other was an impurity (m/z $(M+H)^+$ = 462.3) in 1 to 1 ratio. The impurity was not separated and characterized. Fortunately, under room temperature condition and after 24 h, the desired diamine **130** was obtained exclusively (Scheme 3.10). The main reason for the long reaction time was that the precursor **140** did not completely dissolve in the aqueous methanolic solution. Purification was performed either by silica gel column chromatography (DCM/MeOH) or acid-base extraction. Thus, the highly pure diamine **130** was obtained in 70% overall yield from trityl chloride resin. The viscous, colorless diamine was fully characterized by ^{1}H-NMR, ^{13}C-NMR, 2D-HMQC, HR-MS, IR, LC-MS, elemental analysis and specific optical rotation.

3.3.3 Synthesis of diamine 147

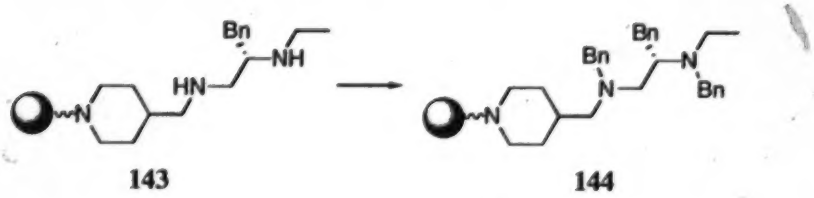
Tertiary diamine **147** could be constructed from the resin bound diamine **135** by direct benzylation or benzoylation followed by reduction. Hall and coworkers[28] reported the benzylation of resin bound polyamines using a 2-bromomethyl phenylboronate ester. In this case, benzyl bromide did not give the fully benzylated product. Several conditions were tried. The best result obtained was a mixture of the desired product **141** with mono benzylation product **142** (6:1) when the benzylation was repeated twice (Scheme 3.11).

The reason for incomplete benzylation could be that the remote nitrogen center was too hindered. To evaluate this hypothesis, resin **143** was constructed using the same protocol as for resin **135**. This time benzylation using benzyl bromide went to completion (Scheme 3.12).



Reaction conditions: resin **135** (1 equiv.), benzyl bromide (10 equiv.), PMP (10 equiv.), THF, 65 °C, 16 h.

Scheme 3.11 Benzylation of resin bound diamine **135** with benzyl bromide

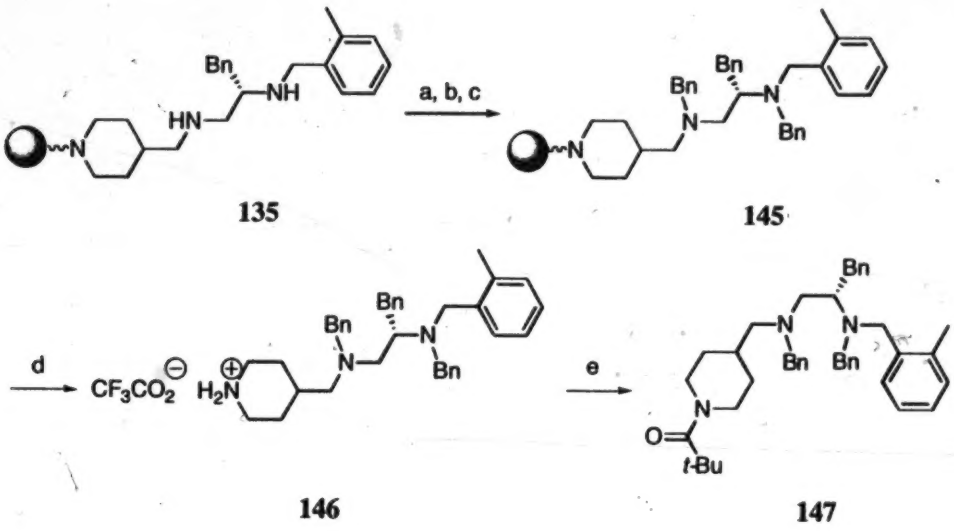


Reaction conditions: resin **143** (1 equiv.), benzyl bromide (10 equiv.), PMP (10 equiv.), THF, 65 °C, 16 h.

Scheme 3.12 Benzylation of resin bound diamine **143** with benzyl bromide

Next, benzoylation of resin **135** was tried followed by reduction. Although the reduction took three days, this strategy worked well and afforded the hindered resin bound tertiary diamine **145** (Scheme 3.13).

The viscous, colorless diamine **147** was obtained (Scheme 3.13), using the same protocol as diamine **130**, in 35% overall yield from trityl chloride resin. Diamine **147** was fully characterized by ^{1}H-NMR, ^{13}C-NMR, 2D-HMQC, HR-MS, IR, LC-MS, and specific optical rotation.



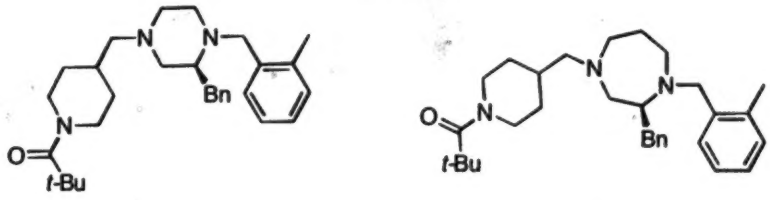
135 145

146 147

Reaction conditions: (a) resin **135** (1 equiv.), benzoyl chloride (10 equiv.), DIPEA (10 equiv.), THF, rt, 16 h. (b) 1M BH_3-THF (20 equiv.), THF, 65 °C, 3 days. (c) piperidine, 65 °C, 16 h. (d) 5% TFA/DCM, 30 min. (e) $(t\text{-BuCO})_2O$ (3 equiv.), Et_3N (3 equiv.), THF, rt, 6 h.

Scheme 3.13 Preparation of diamine **147**

3.3.4 Synthesis of diamine 148 and diamine 149



148 149

Figure 3.4 The structures of diamines **148** and **149**

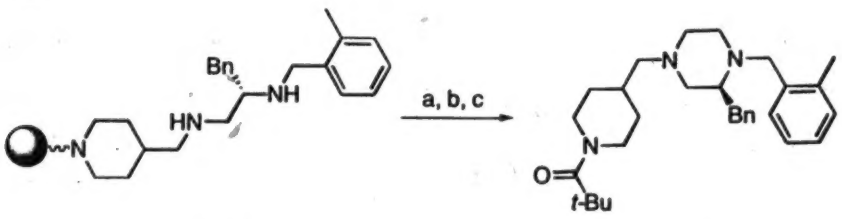
Compound **130** is a linear secondary diamine and **147** is a linear tertiary diamine. Next, it was intended to make tertiary cyclic diamines **148** and **149** (Figure 3.4). As a 7-membered ring adopts a different conformation compared to a 6-membered ring, it was

expected that the resulting ligands could have distinct properties in catalytic asymmetric reactions.

To construct cyclic diamine **148** from resin bound diamine **135**, 1, 2-dibromoethane and 1, 2-diiodoethane were used first, but no desired product formed. It is possible that bromine was not a good leaving group in this situation and 1,2-diiodoethane decomposed during the reaction. Triflate species are widely used as leaving groups. Diol triflates were applied by the Quirion group[29] to form 6-membered rings in the synthesis of 3-substituted 2-oxopiperazines. Hall and coworkers[30] synthesized chiral triazacycloalkane ligands by using diol triflates as reagents and reacting them with resin bound triamines (Scheme 2.16).

Following literature procedures,[31, 32] ethane 1,2-bistriflate and propane 1,3-bistriflates were prepared. ^{1}H-NMR spectra of the crude products were fairly homogeneous and were consistent with literature data.[31]

To form the 6-membered ring, the diluted ethane 1,2-bistriflate solution was added to a resin suspension over 5 h through a syringe pump. After the reaction, LC-MS indicated that the desired compound was generated exclusively, without evidence of cross linked compound and double alkylation by-product. Following the same procedure used for diamine **130**, diamine **148** was obtained in 32% overall yield from trityl chloride resin. (Scheme 3.14).

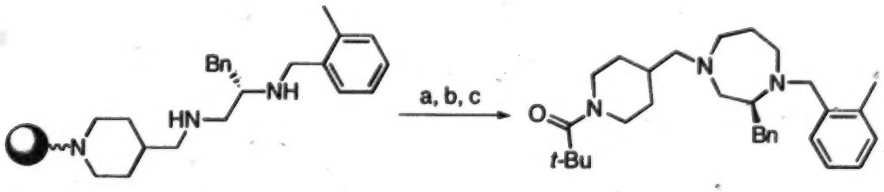


135 **148**

Reaction conditions: (a) resin **135** (1 equiv.). $TfOCH_2CH_2OTf$ (5 equiv), DIPEA (8 equiv.), DCM, 0°C, 5 h, then rt, 10h. (b) 5% TFA/DCM, 30 min. (c) $(t\text{-}BuCO)_2O$ (3 equiv.), Et_3N (3 equiv.), THF, rt, 6 h.

Scheme 3.14 Preparation of diamine **148**

The same procedure as used in the synthesis of **148** generated the 7-membered ring diamine **149** in 22% overall yield from trityl chloride resin. (Scheme 3.15). Both diamines **148** and **149** were obtained as colorless and viscous oils and were characterized by ^{1}H-NMR, ^{13}C-NMR, 2D-HMQC, HR-MS, IR, LC-MS and specific optical rotation [α]. One interesting observation was that the sign of the [α] for the 6-membered ring diamine **148** was positive, compared to the negative sign of its 7-membered ring counterpart diamine **149**. This sign change could be resulting from the different conformations of ring sizes.



135 **149**

Reaction conditions: (a) resin **135** (1 equiv.). $TfO(CH_2)_3OTf$ (5 equiv), DIPEA (8 equiv.), DCM, 0 °C, 5 h, then rt, 10 h. (b) 5% TFA/DCM, 30 min. (c) $(t\text{-}BuCO)_2O$ (3 equiv.), Et_3N (3 equiv.), THF, rt, 6 h.

Scheme 3.15 Preparation of diamine **149**

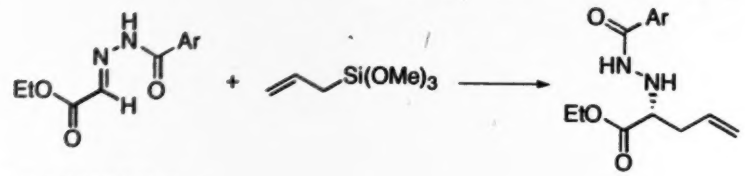
3.4 Preliminary reactions screens using chiral diamines

There are few natural occurring and commercial chiral diamines available in organic synthesis. Chiral diamines **130**, **147**, **148** and **149** could be used as chiral ligands for some asymmetric reactions. Therefore, these potential ligands were tested. The first test reaction was the catalytic asymmetric allylation of hydrazono esters.

3.4.1 Catalytic asymmetric allylation of hydrazono esters

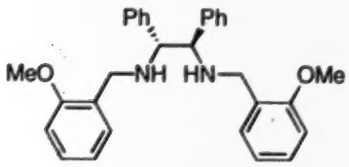
The Kobayashi group[8] has applied chiral diamines such as *N,N'*-(2-methoxylbenzyl)-(1R,2R)-1,2-diphenyl-ethylenediamine **115**, for catalytic asymmetric

allylation of hydrazono esters (Scheme 3.16). The reaction was carried out in aqueous solution and provided a 65~85% ee. It was thought that the ee of this reaction could be improved. Therefore, diamine **130** was employed as a chiral ligand in this reaction.



Ar = 4-MeO-C_6H_4

150 **151**



115

Reaction conditions: hydrazono ester **150** (1 equiv.), allyltrimethoxysilane (3 equiv.), ZnF_2 (20 mol%), ligand (10 mol%), H_2O/THF (V/V 1:9), 0 °C, 55 h.

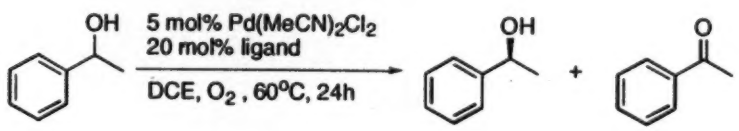
Scheme 3.16 Catalytic asymmetric allylation of hydrazono ester **150**

The reaction was carried out following the literature procedure, but only a 10 % yield was observed, and the product was obtained as a racemic mixture. As a note, a blank reaction (without ligands) and a control reaction (using ligand *N,N'*-(2-methoxylbenzyl)-(1R,2R)-1,2-diphenyl-ethylenediamine) were carried out and the literature data was reproduced. Ligand *N,N'*-(2-methoxylbenzyl)-(1R,2R)-1,2-diphenyl-ethylenediamine and hydrazono ester **150** were prepared following the literature procedure respectively.[8, 33] The ee value of product **151** was determined by chiral HPLC.

3.4.2 Enantioselective palladium (II)-catalyzed aerobic alcohol oxidation

Kinetic resolution is one important method to make chiral compounds. Sigman and coworkers[7, 34] reported that (-)-sparteine was applied in an enantioselective palladium(II) -

catalyzed aerobic alcohol oxidation reaction to form chiral secondary alcohols in moderate yield and 66~99% ee (Scheme 3.17).



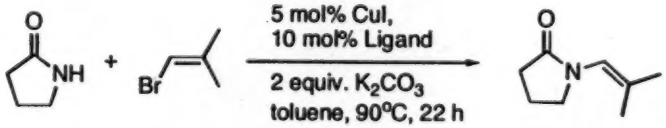
152

Scheme 3.17 Enantioselective palladium (II)-catalyzed aerobic alcohol oxidation

(-)-Sparteine **(114)**, shown in Figure 3.1, is a naturally occurring hindered tertiary diamine. It was thought that diamines **147**, **148** and **149** could coordinate with palladium (II) to form a similar conformation. Therefore, the reaction was carried out using diamines **147**, **148** and **149** respectively. Again, no ee was observed. As a note, the control reaction (using (-)-sparteine) was reproduced and the ee value of product **152** was determined by chiral HPLC.

3.4.3 Copper-catalyzed coupling of amides with a vinyl bromide

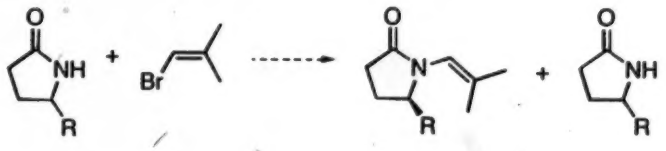
Diamines have also been used for the vinylation of amides.[35] The Buchwald group used one simple diamine, *N,N'*-dimethyl-1,2-ethylene-diamine, to accelerate the vinylation of amides with vinyl halides in 81~95% yield. One of the reactions, using 2-pyrrolidinone as the substrate, is shown in Scheme 3.18.



153 **154** **155**

Scheme 3.18 Copper-catalyzed coupling of amide **153** with vinyl bromide **154**

It was thought that if diamine **130** can catalyse this reaction, it could be used for the kinetic enantioselective resolution of 2-pyrrolidinone derivatives by Cu-catalyzed vinylation (Scheme 3.19).



Scheme 3.19 Possible enantioselective vinylation of 2-pyrrolidinone derivatives

When diamine **130** was used in the reaction, product **155**, *N*-(2-methyl-1-propenyl)-2-pyrrolidinone, was obtained in 27% yield. Although diamine **130** showed a slight activity in this reaction compared to the blank reaction (without any diamine) in which only a trace of product was observed, the yield of this reaction was still low. Hence, substituted 2-pyrrolidinone derivatives were not used for further investigation.

From the results of a limited test set of three reactions, it can be seen that diamine **130**, **147**, **148** and **149** did not induce chirality.

3.5 Summary

In this project, four different chiral diamines, including a linear secondary diamine **130**, a linear tertiary diamine **147**, as well as 6-membered and 7-membered tertiary diamines **148**, **149**, were synthesized and fully characterized. The trifluoroacetyl group was used to temporarily mask the resin bound secondary amine to form the linear secondary diamine. Diol bistriflates were used for the cyclization to form cyclic tertiary diamines. Although no enantiomeric excess value was observed when these diamines were tested for two asymmetric reactions, it is clear that a large variety of chiral diamines could be synthesized using this general method and tested for asymmetric reactions in the future.

3.6 Experimental section

3.6.1 General

See Section 2.5.1 (Chapter 2).

Optical rotations were measured on a PERKIN-ELMER 241 polarimeter.

Reverse phase chiral HPLC:

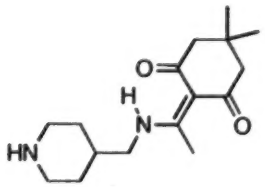
Zobax OD-RH reverse chiral column

Mobile phase: A: acetonitrile (40%), B: water (60%)

Flow rate: 0.5 mL/min; Injection: 2 µl; Temp: 25 °C

Detector: UV DAD at 210 and 254 nm

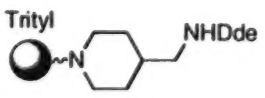
3.6.2 Synthesis of 4-(Dde-aminomethyl)piperidine 133



To 2.26 g (19.8 mmol) of 4-(aminomethyl) piperidine in 15 mL THF, was added 7.79 g 2-acetyldimedone (20.8 mmol) in 15 mL THF. After 6 h stirring at room temperature, the solvent was evaporated providing a yellow solid. The crude compound **133** was used to load to trityl chloride resin without further purification.

LC-MS: reverse phase gradient elution method 1 (Chapter 2, Section 2.5.1.2). Rt = 7.498 min. % purity: 96%. ES-MS *m/z* $(M+H)^+$ = 279.2.

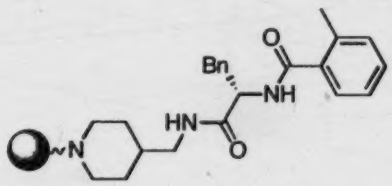
3.6.3 Synthesis of resin 134



1.94 g trityl chloride resin (0.88 mmol/g) was swelled and suspended with 25 mL DCM in a PP vessel. To this suspension was added a solution containing 950 mg 4-(Dde-aminomethyl)piperidine (3.4 mmol) and 587 μL DIPEA (3.4 mmol) in 5 mL DCM. The suspension was shaken 2 h at room temperature, then drained and washed by DCM (2x), MeOH (2x), DCM/MeOH/DEPEA (17:2:1) (2x), THF (3x), MeOH (3x), DCM (3x). Negative result of Kaiser test was observed.

LC-MS: reverse phase gradient elution method 1 (Chapter 2, Section 2.5.1.2), Rt = 7.463 min. % purity > 95%. *m/z* $(M+H)^+$ = 279.2.

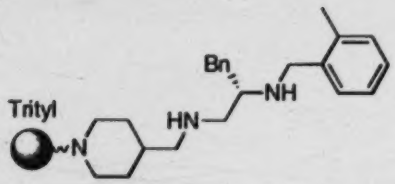
3.6.4 Synthesis of resin 132



10 mL 2% NH_2NH_2/DMF was added to the PP vessel, which was charged with 1.0 g resin **134** followed by shaking for 10 min. The solution was drained. Another 10 mL fresh 2% NH_2NH_2/DMF was added, and the mixture was shaken for 30 min. The resin was washed with DMF, THF, and MeOH 3 times each. LC-MS indicated the Dde group has been removed. Using Fmoc AA coupling procedure (Chapter 2, Section 2.5.1.3), the resulting resin was coupled with L-phenylanaline, and then *o*-toluenic acid to generate resin **132**.

LC-MS: reverse phase gradient elution method 1 (Chapter 2, Section 2.5.1.2), Rt = 8.919 min. % purity > 95%. *m/z* $(M+H)^+$ = 380.2.

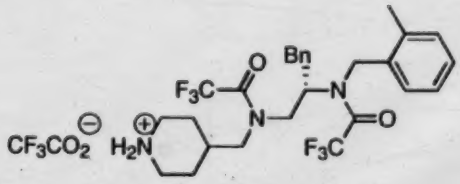
3.6.5 Synthesis of resin bound diamine 135



The general reduction procedure (Chapter 2, Section 2.5.1.5) of resin **132** by 1M BH_3-THF generated resin bound diamine **135**. The new loading level of resin bound diamine was 0.65 mmol/g, which based on the percentage (2.71%) of nitrogen in the resin by elemental analysis.

LC-MS: reverse phase gradient elution method 1 (Chapter 2, Section 2.5.1.2), Rt =9.320 min. % purity > 95%. *m/z* $(M+Na)^+$ = 376.2.

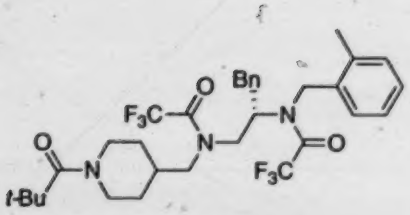
3.6.6 Synthesis of 139



0.7 mmol resin in PP vessel was swollen in dry DCM (15 mL) for 30 min, then washed with dry THF (2x), suspended in 15 mL dry THF under nitrogen. To the suspension was added 10 equivalents of pyridine, followed by dropwise addition of trifluoroacetic anhydride at room temperature in 10 min under nitrogen while shaking the vessel. The PP vessel was capped and shaken for 2.5 h at room temperature. The resultant slight orange resin was washed with THF, MeOH and DCM three times each. Kaiser test of the resin was negative. Cleavage was performed under standard cleavage condition by using 5% TFA.

LC-MS: reverse phase gradient elution method 1 (Chapter 2, Section 2.5.1.2), Rt = 11.875 min. % purity > 95%. *m/z* $(M+H)^+$ = 544.2.

3.6.7 Synthesis of 140



0.2513 g TFA salt (0.38 mmol) was dissolved in dry THF in a round bottom flask, and then cooled to 0 °C. To the solution was added 160 μL Et_3N (3 equiv.) following dropwise addition of 232 μL pivalic anhydride (3 equiv.) in 2 mL dry THF at 0 °C. The solution was stirred overnight at room temperature, then concentrated to provide a yellow slurry of crude compound **140**. The EtOAc solution of the crude product was washed sequentially with 5% aqueous $NaHCO_3$, 1 N HCl, H_2O and brine, dry over Na_2SO_4. Silica gel flash column chromatography (Hexane/EtOAc: 4:1 to 2:1) generated 0.1700 g compound **140** (72% yield).

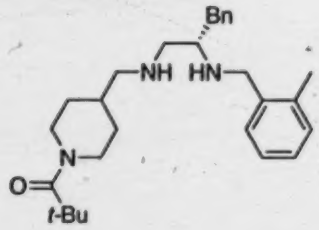
TLC: R_f= 0.43 (EtOAc/Hexane 1:1).

LC-MS: reverse phase gradient elution method 1 (Chapter 2, Section 2.5.1.2), Rt = 15.307 min, *m/z* $(M+H)^+$ = 628.3, % purity > 98%.

HR-MS: for $C_{32}H_{39}N_3O_3F_6$: *m/z* $(M+H)^+$ calcd. 628.29684, found: 628.29687.

Elemental analysis: for $C_{32}H_{39}N_3O_3F_6$ calcd. C 61.23, H 6.26, N 6.69; found: C 61.00, H 6.40, N 6.53.

3.6.8 Synthesis of diamine 130



To a round bottom flask charged with 0.43 g compound **140**, was added 10 mL 7% K_2CO_3 in aqueous methanol (v/v = 2:5) at room temperature, then stirred. The reaction was monitored by TLC and was complete in 24 h, Evaporation of solvents under reduced pressure provided crude compound **130**. The crude compound was re-dissolved in 10 mL H_2O, and extracted with DCM (3 x 10mL). The combined DCM extract was washed with H_2O, brine and dried over Na_2SO_4. A colorless viscous compound **130** was obtained after evaporation and dried over high vacuum. After purification by silica gel flash column chromatography (DCM : MeOH = 16:1 to 8:1), 0.299 g pure colorless viscous **130** was obtained (95% yield from **140**, corresponding to 70% yield from trityl resin).

TLC: R_f = 0.42 (DCM : MeOH = 8:1).

IR(DCM cast): 1626, 2920, 3024, 3311cm^{-1}.

^1H NMR (500 MHz, CD_3OD) δ(ppm): 0.95-1.05 (m, 2H), 1.58-1.70 (m, 3H), 1.25 (s, 9H), 2.25 (s, 3H), 2.27 (dd, 6.5, 11.8 Hz, 1H), 2.31 (dd, 6.6, 11.8 Hz, 1H), 2.46 (dd, 7.9, 12.0 Hz, 1H), 2.58 (dd, 3.9, 12.0 Hz, 1H), 2.65 (dd, 9.4, 15.7 Hz, 1H), 2.77 (app. t, 11.7 Hz, 2H), 2.88-2.95 (m, 2H), 3.68 (d, 12.9 Hz, 1H), 3.79 (d, 12.9 Hz, 1H), 4.32 (br. s, 1H), 4.35 (br. s, 1H), 7.10-7.14 (m, 3H), 7.17-7.22 (m, 4H), 7.25-7.30 (m, 2H).

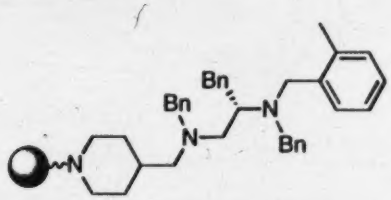
^{13}C NMR (100 MHz, CD_3OD) δ(ppm): 19.1 (CH_3), 28.8 (CH_3), 31.80 (CH_2), 31.82 (CH_2), 37.4 (CH), 39.8 (C), 40.3 (CH_2), 46.5 (CH_2), 46.6 (CH_2), 50.0 (CH_2), 53.9 (CH_2), 56.1 (CH_2), 59.2 (CH), 126.9 (CH), 127.4 (CH), 128.4 (CH), 130.31 (CH), 130.33 (CH), 131.4 (CH), 137.6 (C), 139.1 (C), 140.5 (C), 178.3 (CO).

LC-MS: reverse phase gradient elution method 2 (Chapter 2, Section 2.5.1.2), Rt = 9.669 min. %purity > 99%. *m/z* $(M+1)^+$ = 436.3.

HR-MS for $C_{28}H_{42}N_3O_1$: *m/z* $(M+H)^+$ calcd. 436.332788 found 436.332334.

$[\alpha]^{25}_D$ = 17.31° (*c* = 0.07, $CHCl_3$).

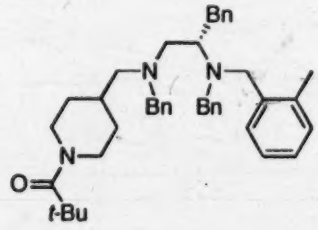
3.6.9 Synthesis of compound 145



Resin **135** in a PP vessel was swollen in DCM, then washed with dry THF three times followed by suspension into dry THF under nitrogen. To the suspension was added 10 equivalents of DIPEA followed by 10 equivalents of benzoyl chloride at room temperature and under nitrogen. The PP vessel was sealed and shaken overnight. The resin was drained and washed with THF, MeOH and DCM three times each, followed by drying over high vacuum. LC-MS indicated that the resin was fully protected. The standard procedure for reduction of the resultant resin with 1M BH_3-THF (Chapter 2, Section 2.5.1.5) for 3 days provided resin bound linear tertiary diamine **145**.

LC-MS: reverse phase gradient elution method 1 (Chapter 2, Section 2.5.1.2), Rt =12.316 min. % purity > 95%. *m/z* $(M+H)^+$ = 532.4.

3.6.10 Synthesis of tertiary diamine 147



The resin **145** was cleaved by 5% TFA/DCM. After evaporation, the residue was further dried under high vacuum to give the TFA salt as a slight yellow solid. The TFA salt was used for next step without further purification. The TFA salt reacted with pivalic anhydride using the same procedure as the synthesis of compound **140** provided crude

compound **147**. Pure colorless viscous **147** was obtained by silica gel flash column chromatography (Hex : EtOAc = 3:1 to 2:1).

TLC: R_f= 0.37 (Hex : EtOAc 2:1).

IR (DCM cast film): 1627, 2849, 2925, 3025cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 0.72 (ddd, 3.4, 12.2, 12.2 Hz, 1H), 0.89 (ddd, 3.4, 12.2, 12.2 Hz, 1H), 1.29 (s, 9H), 1.48-1.58 (m, 1H), 1.63 (d, 12.2 Hz, 1H), 1.72 (d, 12.3 Hz, 1H), 2.10 (s, 3H), 2.10 (dd, 5.0, 12.8 Hz, 1H), 2.16 (dd, 5.7, 12.8 Hz, 1H), 2.49 (dd, 8.2, 12.6 Hz, 1H), 2.61-2.71 (m, 2H), 2.75 (dd, 4.1, 12.7 Hz, 1H), 2.77 (dd, 8.7, 13.9 Hz, 1H), 2.92 (dd, 5.5, 13.9 Hz, 1H), 3.07 (m, 1H), 3.35 (d, 13.6 Hz, 1H), 3.62 (d, 13.8 Hz, 1H), 3.65 (d, 13.5 Hz, 1H), 3.65 (s, 2H), 3.73 (d, 13.7 Hz, 1H), 4.19 (br. d, 12.0 Hz, 1H), 4.28 (br. d, 12.8 Hz, 1H), 7.02-7.32 (m, 19H).

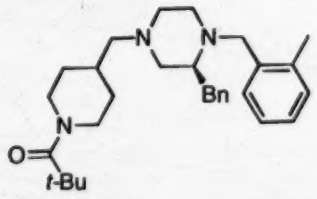
^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm): 19.3 (CH_3), 28.4 (CH_3), 30.8 (CH_2), 31.0 (CH_2), 34.5 (CH), 35.9 (CH_2), 38.7 (C), 45.0 (CH_2), 45.2 (CH_2), 51.1 (CH_2), 54.0 (CH_2), 55.4 (CH_2), 58.3 (CH), 60.5 (CH_2), 61.1 (CH_2), 125.6 (CH), 125.8 (CH), 126.6 (CH), 126.8 (CH), 126.9 (CH), 128.0 (CH), 128.1 (CH), 128.9 (CH), 129.0 (CH), 129.1 (CH), 129.6 (CH), 130.1 (CH), 136.8 (C), 137.3 (C), 139.7 (C), 139.9 (C), 141.1 (C), 176.0 (CO).

LC-MS: reverse phase gradient elution method 2 (Chapter 2, Section 2.5.1.2), Rt = 13.155 min, % pure > 95%. *m/z* (M+1)$^+$ = 616.4.

HR-MS for $C_{42}H_{54}N_3O$ *m/z* (M+H)$^+$ calcd. 616.42614, found 616.42654.

$[\alpha]^{25}_D$ = (-47.32°) (*c* = 0.012, $CHCl_3$).

3.6.11 Synthesis of cyclic tertiary diamine 148



To the suspension of resin **135** in DCM in a round bottom flask, was added 8 equivalents of DIPEA (respective to the resin), followed by cooling the suspension to 0

°C. This was followed by slow addition (through a syringe pump) of 5 equivalents of ethane 1,2-bistriflate (respective to the resin) during 5 h, and then the suspension was stirred at room temperature overnight. The resin was washed with DCM, THF, MeOH three times each, and dried under high vacuum. The resultant resin was cleaved by 5% TFA/DCM. After DCM was evaporated, the residue was further dried under high vacuum to give a slight yellow solid TFA salt. The TFA salt reacted with pivalic anhydride using the same procedure as the synthesis of compound **140** provided crude compounds **148**, which was purified by silica gel flash column chromatography (Hex : EtOAc = 8:1 to 4:1). Diamine **148** was slightly yellow and fairly viscous and obtained in 32% yield from resin **135**.

TLC: R_f= 0.30 (Hex: EtOAc = 4:1).

IR (DCM cast film): 1628, 2924, 3023cm^{-1};

^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.02-1.11 (m, 2H), 1.28 (s, 9H), 1.58-1.69 (m, 1H), 1.76 (d, 13.0 Hz, 1H), 1.82 (d, 13.0 Hz, 1H), 2.02 (dd, 7.0, 12.2 Hz, 1H), 2.09 (dd, 7.5, 12.2 Hz, 1H), 2.14-2.26 (m, 2H), 2.26-2.33 (br. m, 1H), 2.38 (s, 3H), 2.33-2.48 (m, 2H), 2.68-2.82 (m, 4H), 2.83-2.92 (br. t, 1H), 3.06 (d, 11.7 Hz, 1H), 3.50 (d, 12.0 Hz, 1H), 3.94 (d, 12.5 Hz, 1H), 4.39 (br. s, 2H), 7.10-7.35 (m, 9H).

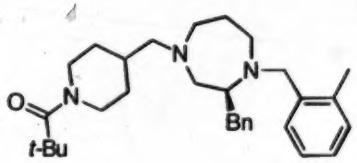
^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm): 19.3 (CH_3), 28.5 (CH_3), 31.1 (CH_2), 31.3 (CH_2), 33.8 (CH), 38.7 (C), 45.28 (CH_2), 45.36 (CH_2), 45.39 (CH_2), 53.7 (CH_2), 56.7 (CH_2), 60.3 (CH), 64.5 (CH_2), 125.5 (CH), 125.8 (CH), 126.9 (CH), 128.3 (CH), 129.2 (CH), 129.6 (CH), 130.3 (C), 137.1 (C), 137.5 (C), 140.5 (C), 176.1 (CO).

LC-MS: reverse phase gradient elution method 2 (Chapter 2, Section 2.5.1.2), Rt = 13.780 min, %purity > 98%. *m/z* $(M+1)^+$ = 462.3.

HR-MS for $C_{30}H_{43}N_3O$: *m/z* $(M+H)^+$ calcd. 462.34789, found 462.34757.

$[\alpha]^{25}_D$ = + 49.51° (*c* =0.012, $CHCl_3$)

3.6.12 Synthesis of cyclic tertiary diamine 149



The same procedure of the synthesis of **148** was followed to generate slight yellow and viscous compound **149** in 22% yield from resin **135**, except 1,3-diol bistriflate was used for cyclization,

TLC: $R_f = 0.24$ (Hex : EtOAc = 1:1)

IR (DCM cast): 1628, 2924, 3023, 3059cm^{-1};

^1H NMR (400 MHz, $CDCl_3$) δ(ppm): 0.98-1.10 (m, 2H), 1.29 (s, 9H), 1.44-1.54 (m, 1H), 1.55-1.65 (m, 1H), 1.69 (d, 13.1 Hz, 1H), 1.75-1.90 (m, 2H), 2.20 (dd, 8.1, 12.4 Hz, 1H), 2.26 (dd, 6.4, 12.4 Hz, 1H), 2.31 (s, 3H), 2.45 (dd, 7.2, 13.8 Hz, 1H), 2.52-2.78 (m, 7H), 2.85 (dd, 5.3, 13.4 Hz, 1H), 2.90-3.04 (m, 2H), 3.78 (d, 12.2 Hz, 1H), 3.86 (d, 12.2 Hz, 1H), 4.36 (br. s, 1H), 4.39 (br. s, 1H), 7.06-7.34 (m, 9H).

^{13}C NMR (125 MHz, $CDCl_3$) δ(ppm): 19.2 (CH_3), 28.5 (CH_3), 27.0 (CH_2), 31.10 (CH_2), 31.14 (CH_2), 35.2 (CH), 38.6 (CH_2), 38.7 (C), 45.3 (CH_2), 45.4 (CH_2), 48.8 (CH_2), 55.9 (CH_2), 56.6 (CH_2), 58.5 (CH_2), 64.5 (CH), 65.1 (CH_2), 125.4 (CH), 125.7 (CH), 126.7 (CH), 128.1 (CH), 129.2 (CH), 129.5 (CH), 130.2 (CH), 137.2 (C), 138.2 (C), 140.8 (C), 176.1 (CO).

LC-MS: reverse phase gradient elution method (Chapter 2, Section 2.5.1.2, Rt = 9.170 min, %puriety > 98%, *m/z* $(M+H)^+ = 476.4$.

HR-MS for $C_{31}H_{45}N_3O$: *m/z* $(M+H)^+$ calcd. 476.36354, found: 476.36351.

$[\alpha]^{25}_D = -21.06°$ (c = 0.013, $CHCl_3$).

3.7 Bibliography

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Chapter 4 General Conclusions

Two projects, the synthesis of exo-peptides (acetylated polyamines), and the synthesis of chiral diamines, were discussed in this thesis.

4.1 Summary, conclusions, and future directions with exo-peptides

Design, synthesis and studies of unnatural biopolymers are important ways for humans to understand the natural world, and eventually provide in the benefits of human diseases treatment, such as HIV and cancer therapies.

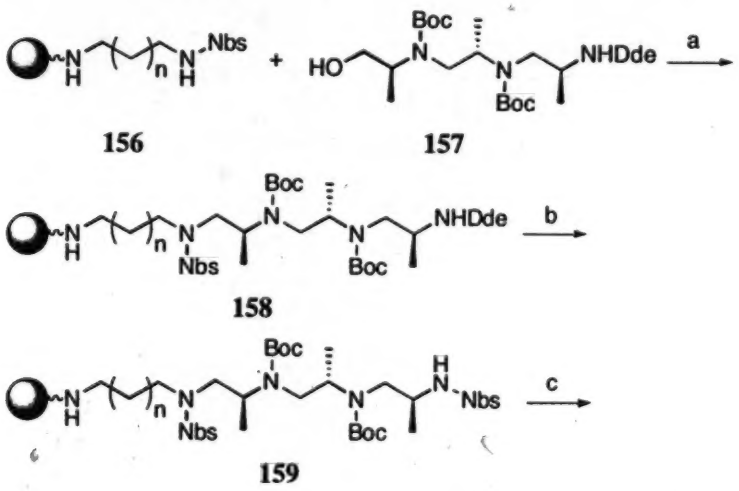
To develop a route to exo-peptides, both solid phase and solution phase approaches were employed. For solid phase approaches, it was found that the C-O ether bond within linkers of a variety of resins, including trityl chloride resin, Wang resin, DHP resin, silyl resins and diphenyldiazomethane resin, was not tolerant to the borane tetrahyrofuran reduction conditions. As a result, most compounds were released prematurely from the resin during the reduction. No desired compound was observed when Fmoc-alaninol was utilized as a Mitsunobu reaction substrate with resin bound sulfonyl amide **57**. These results showed that the Mitsunobu reaction strategy was not a practical approach. The linear strategy, construction of long peptides on resin followed by cleavage, reduction and acetylation in solution phase, was halted because of aggregation problems resulting in incomplete coupling upon six-unit peptides. The fragment strategy, i.e. the repeating coupling of three units, followed by reduction and protection, was not successful due to a lack of proper amine protecting group. Trityl, Nbs and Boc protecting groups were tested, and only the Boc group afforded full protection of resin bound polyamine **65**. Afterwards, it was found that over reduction of Boc protected compounds resulted in the formation of undesired products.

The synthesis of long peptides, followed by reduction and acetylation in solution phase, was only successful in the synthesis of an octa-alanine peptide. Low solubility of the octa-alanine peptide contributed to problems for further amide bond extension. Based on those efforts, the synthesis of exo-peptides has proved to be very challenging.

To eventually synthesize exo-peptides, the Hmb amino acids could be an alternative method. Insertion of Hmb amino acids in every 6 position of peptides is known to

interrupt inter- and intrachain interaction, which is believed to be the major reason for aggregation. As mentioned in Section 2.3.1.4 (Chapter 2), only a few Hmb amino acids are available, which means that only a few types of exo-peptides could be synthesized by using Hmb amino acids. Also, these types of reagents are quite expensive. Except for the limitations and cost, one additional challenge could be the reduction and acetylation of long polyamines in solution phase, although the model test of short peptides, with three and four amide bonds, worked efficiently. Increasing reduction time and using strong reducing reagent, such as $LiAlH_4$, could work for longer peptides, but, the use of $LiAlH_4$ could cause purification problems.

The strategy using the Mitsunobu reaction is still possible. Combining those positive results from this thesis, a possible revised approach is shown in Scheme 4.1. For this approach, the key step would be the Mitsunobu reaction.

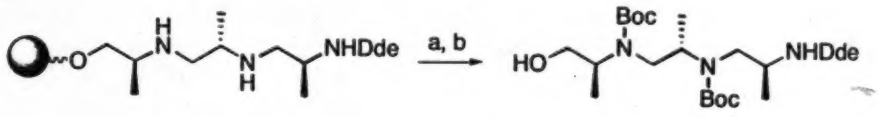


Reaction conditions: (a) Mitsunobu reaction conditions. (b) (i) 2% NH_2NH_2 in DMF. (ii) Nbs-Cl, Et_3N. (c) repeat steps (a) and (b).

Scheme 4.1 Possible approach to prepare resin bound polyamines

Fmoc-alaninol did not give the desired product in the Mitsunobu reaction (Chapter 2, Scheme 2.33). The reason could be the steric hindrance of the Fmoc group or the

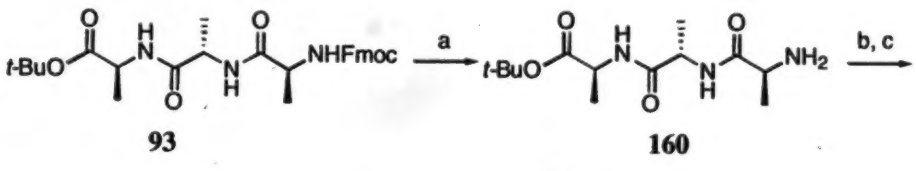
methyl group, or a combination of both. If it is the hindrance of the Fmoc group, then terminal Fmoc-protected long polyamine alcohols could give the desired products. Alcohol **157** could be prepared from resin bound amine **24** on solid support (Scheme 4.2) or from **93** in solution phase (Scheme 4.3).



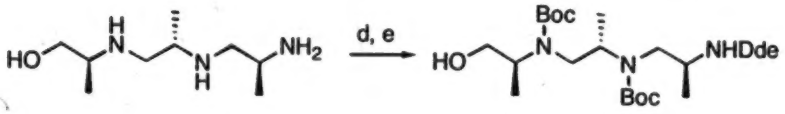
24 **157**

Reaction conditions: (a) 5% TFA/DCM. (b) $(t\text{-BuOCO})_2O$, DIPEA.

Scheme 4.2 Possible approach to prepare compound **157** on solid support



93 **160**



157

Reaction conditions: (a) Et_2NH. (b) BH_3-THF reduction. (c) work up. (d) Dde-OH. (e) DIPEA, $(t\text{-BuOCO})_2O$.

Scheme 4.3 Possible approach to prepare compound **157** in solution phase

The Boc group afforded the fully protected polyamine **65** on solid phase, so, the protection step in solution phase should be easier. Perhaps some mono protected by-product, or carbonic acid *tert*-butyl ester could form, but purification by flash column chromatography would provide the pure protected polyamine alcohol **157**.

4.2 Summary, conclusions, and future directions with chiral diamines

Chiral ligands are crucial in asymmetric reaction systems. Chiral diamines and their derivatives are one type of important chiral ligand, but have limited availability. In this project, a general method for the synthesis of chiral diamines was developed.

The resin bound linear secondary polyamine **135** was constructed using Fmoc AA coupling method, followed by BH_3-THF reduction. Diamine **130** was made from resin **135**, in which the trifluoroacetyl group was used to temporarily mask the secondary amine. A hindered linear diamine **147** was achieved by double reduction of the benzoyl amide. Cyclic diamines **148** and **149**, containing 6- and 7-membered rings respectively, were generated by treatment of resin **135** with diol triflates. All of these diamines were purified by column chromatography. It is anticipated that this general method can be used in the synthesis of a library of unnatural chiral diamines. When these ligands were tested for the two asymmetric reactions, no enantioselectivity was observed. However, one has to mention that there are many more reactions that could be carried out using these chiral diamines as ligands. Furthermore, using our protocol, a variety of these type of ligands can be synthesized and screened in many other asymmetric reactions.

Indeed, it is quite common to make a library of ligands to screen reactions. In this case (Figure 4.1), the R^1 group could come from different amino acids and the aromatic group could accommodate different substituents. Hence, a variety of diamines, including linear secondary and tertiary diamines as well as cyclic tertiary diamines would be available for screening in these reactions.

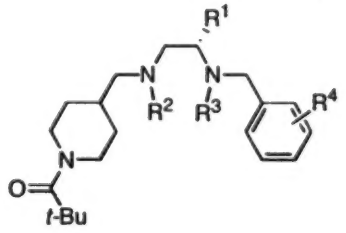
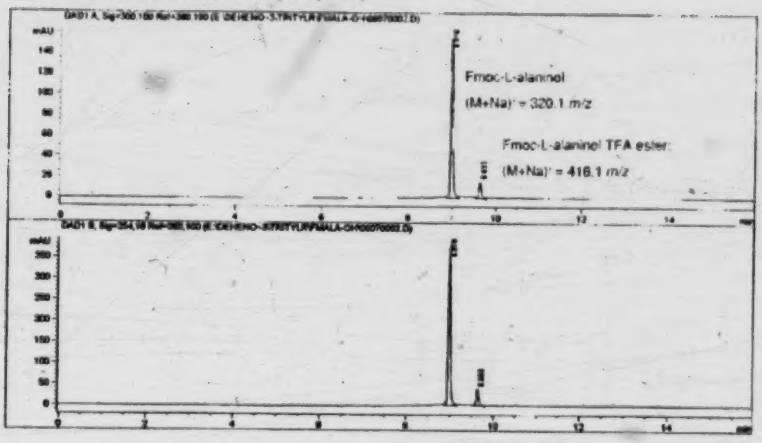
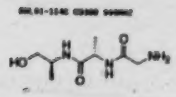


Figure 4.1 General structures of chiral diamines

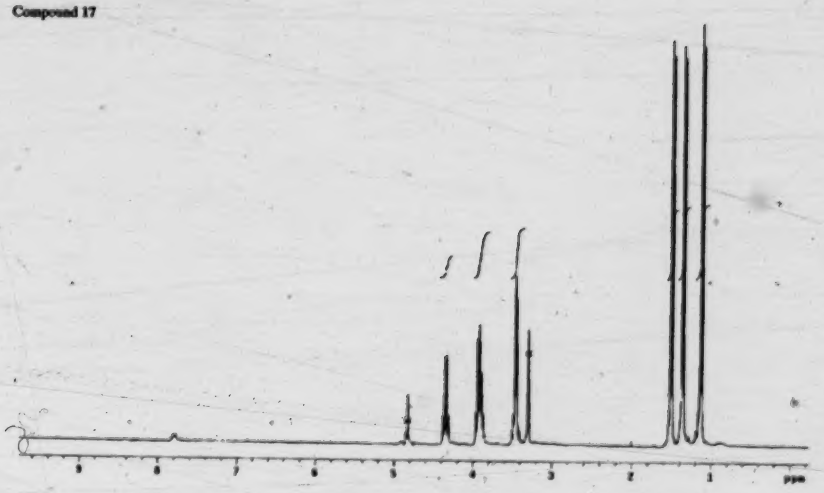
Appendix of selected spectra

Compound 23 Fmoc-L-alaninol (cleaved from trityl resin)

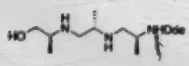


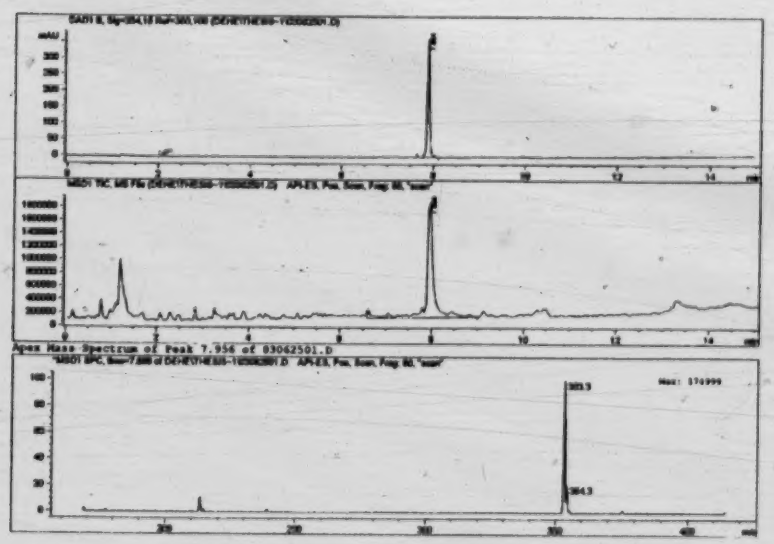


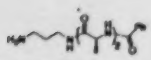
Compound 17



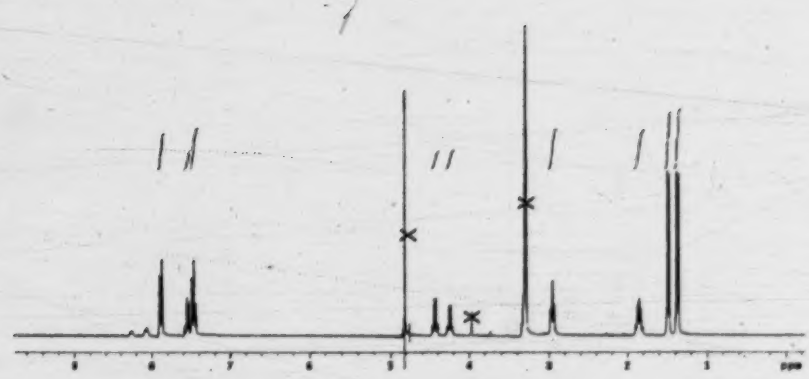
Compound 24 -

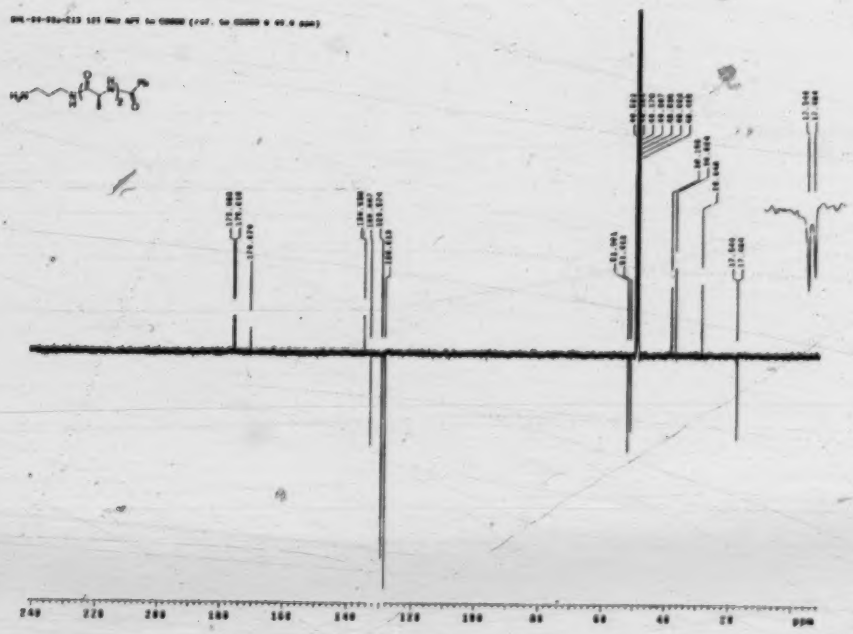


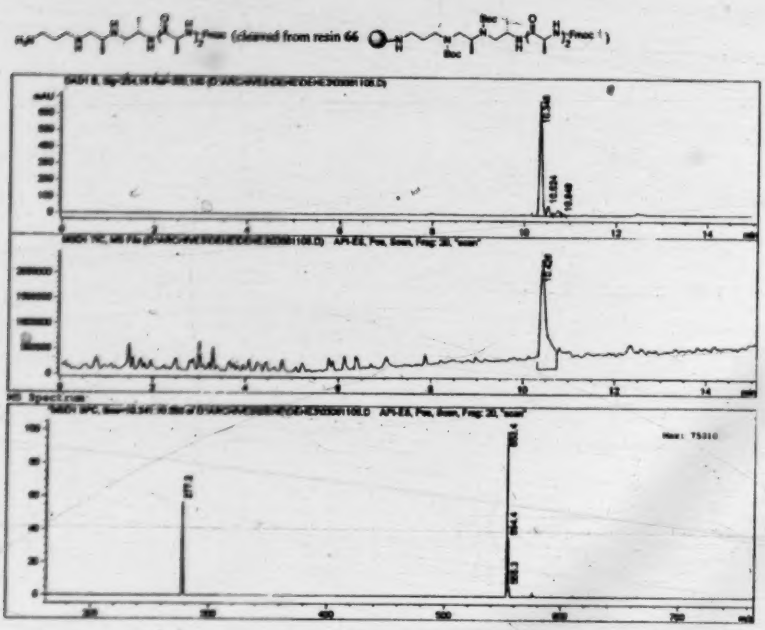




Compound 78



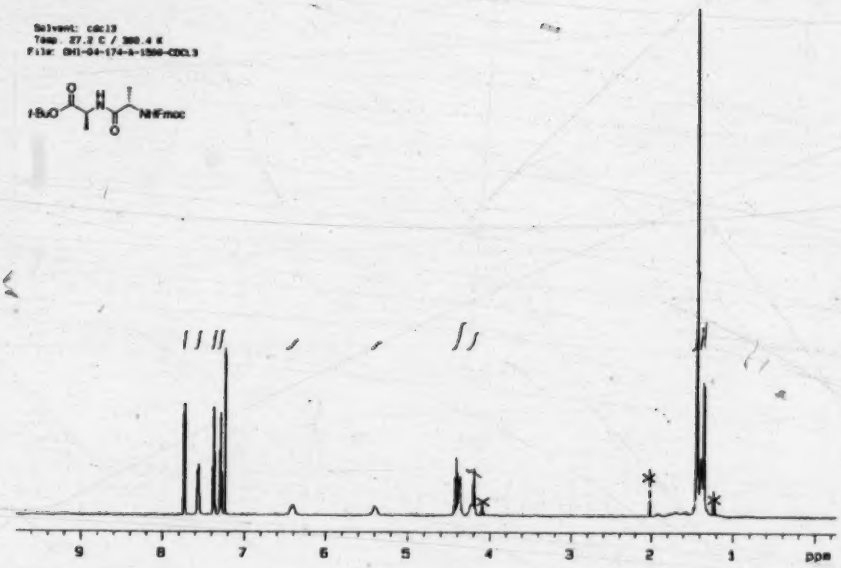




Compound 98

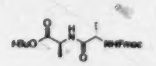
DH1-04-174-A

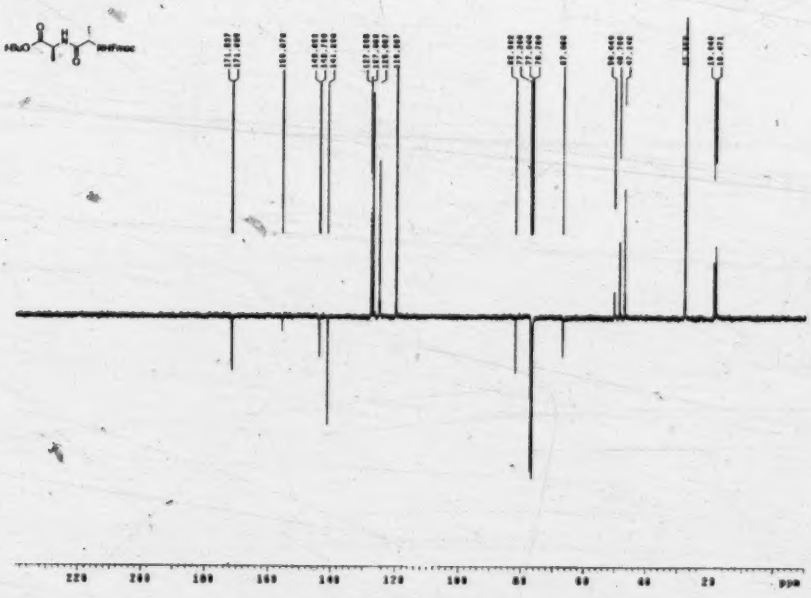
Solvent: cdcl3
Temp: 27.8 C / 300.4 K
File: DH1-04-174-A-1H-CDCL3



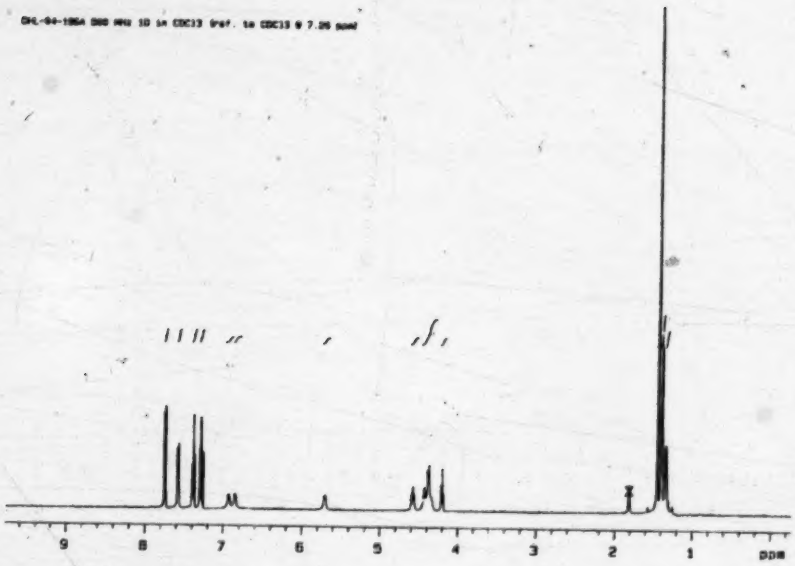
Compound 98

DH1-04-174-A1-C13 125 MHz APT in CDCl3

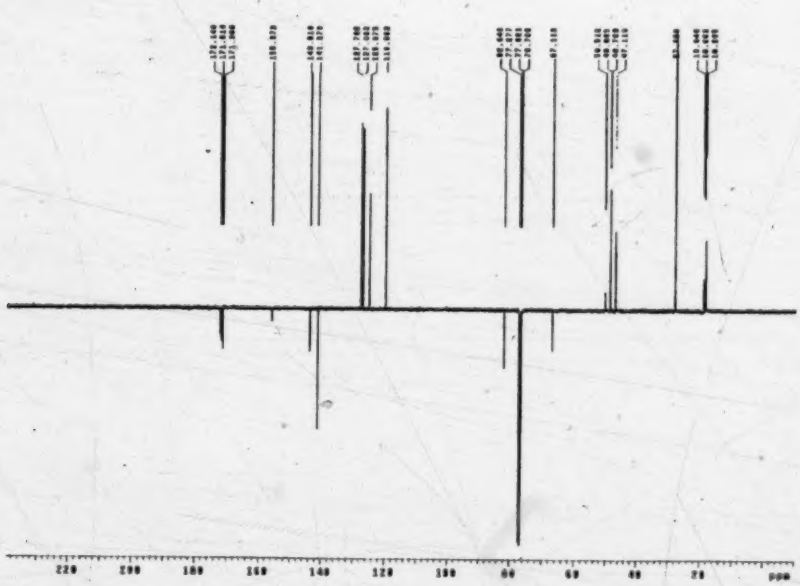




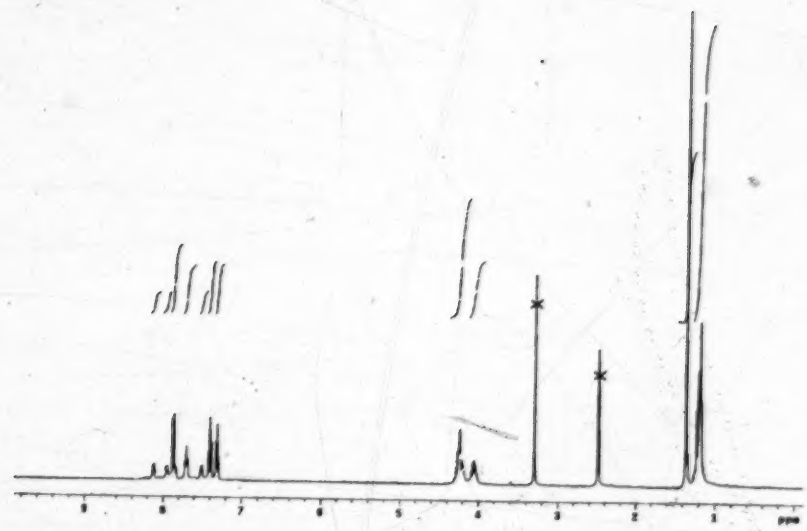
Compound 93 t-BuO-(Ala)$_3$-Fmoc



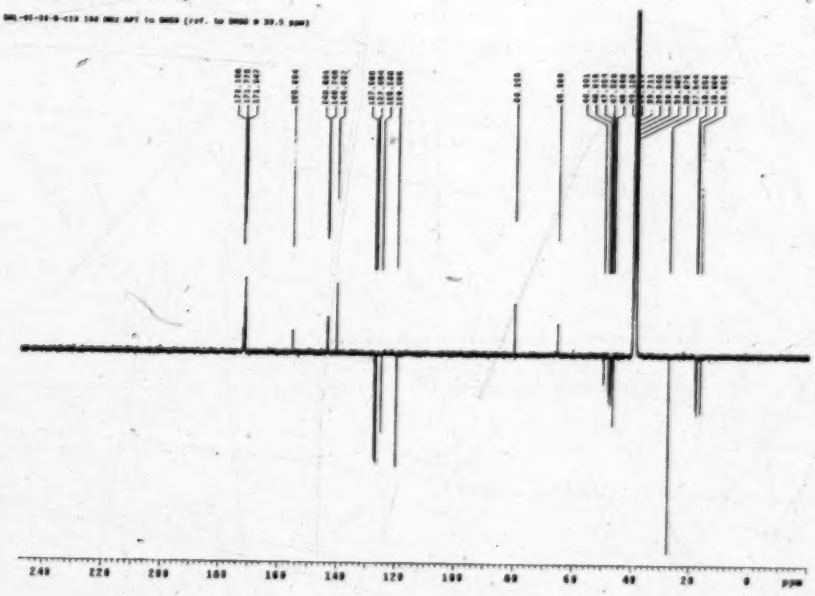
Compound 93 t-BuO-(Ala)$_3$-Fmoc



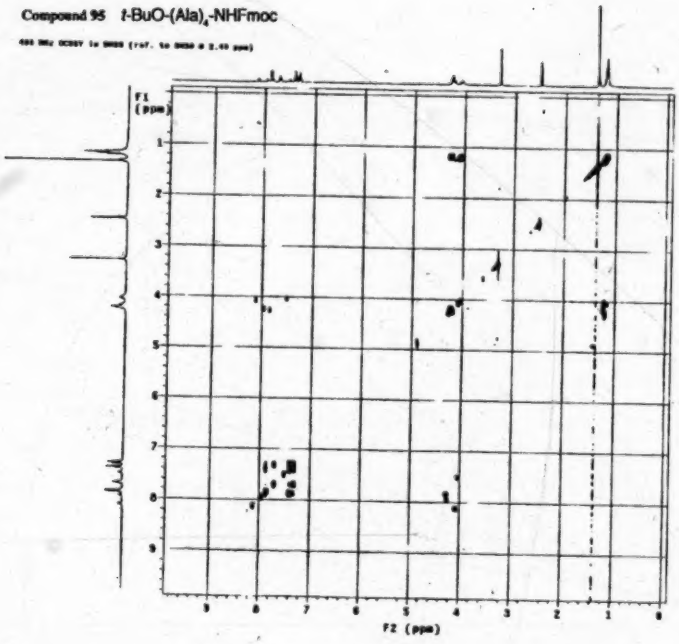
Compound 95 *t*-BuO-(Ala)$_4$-NHFmoc



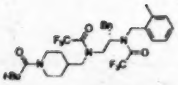
Compound 95 *t*-BuO-(Ala)$_4$-NHFmoc

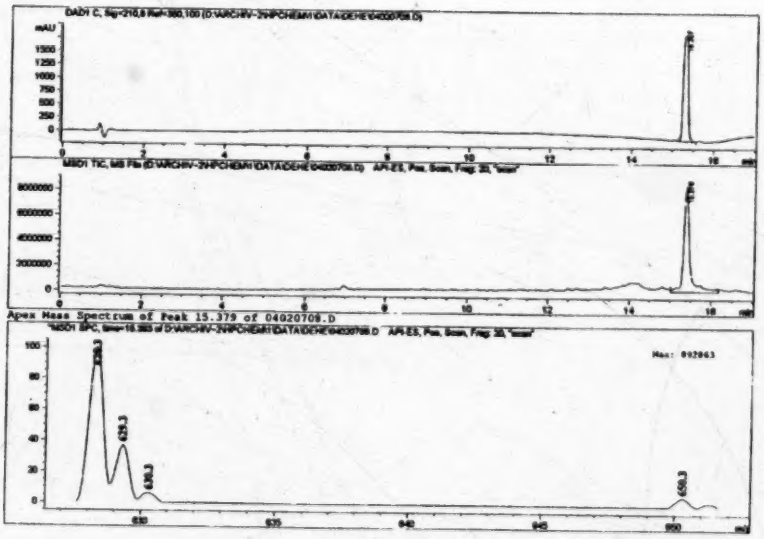


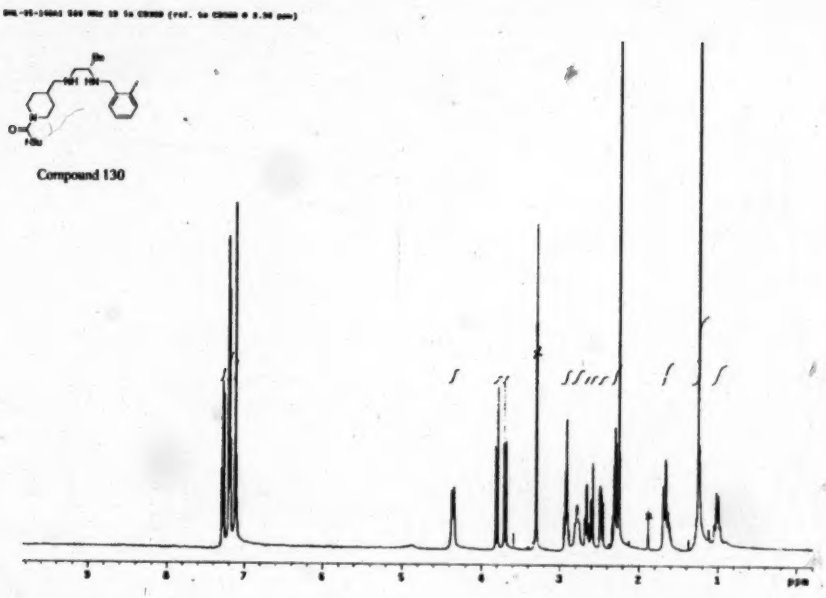
Compound 95 *t*-BuO-(Ala)$_4$-NHFmoc



Compound 140







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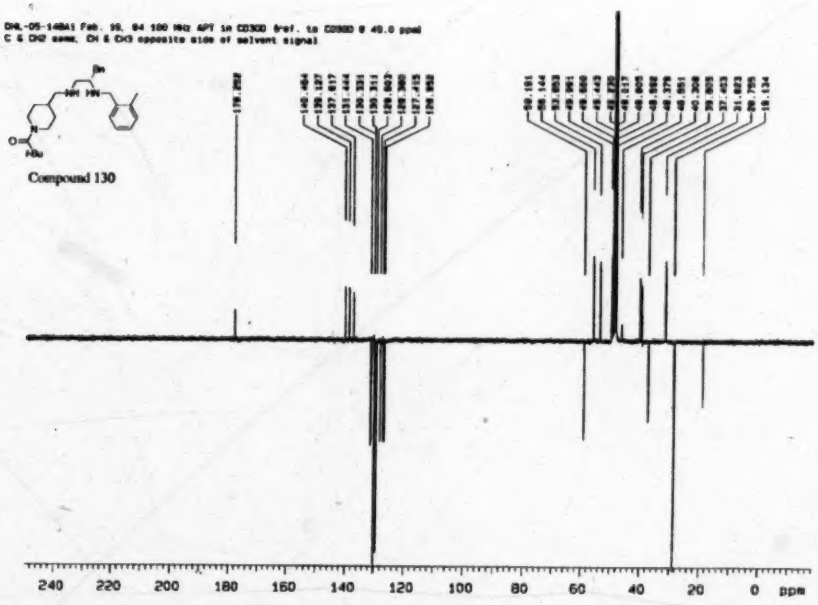

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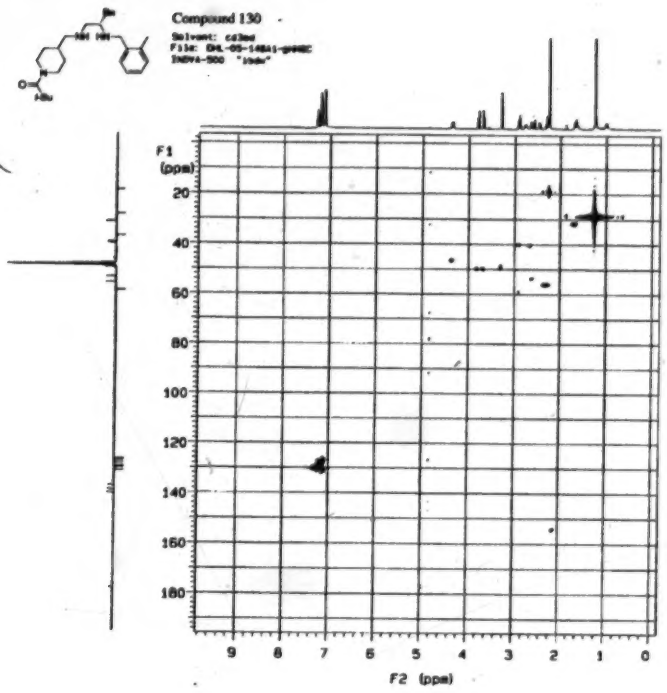

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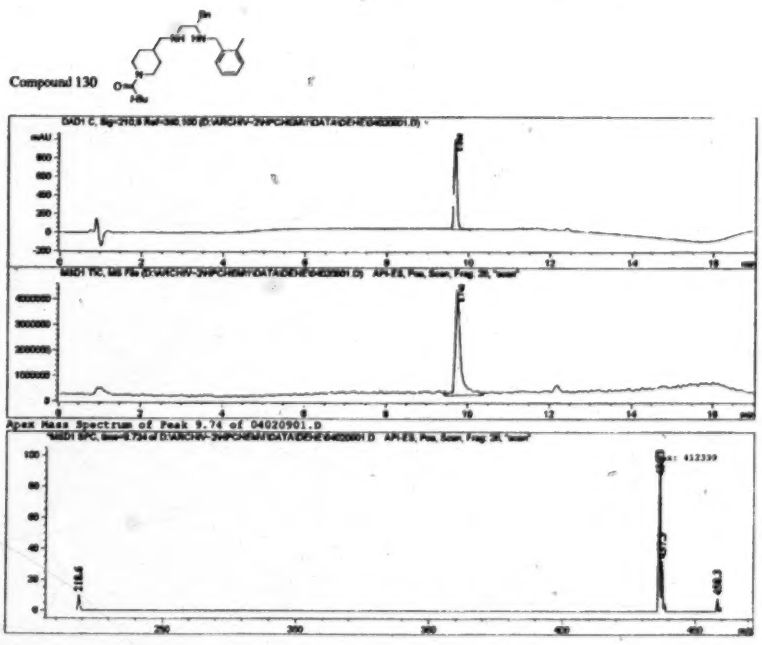

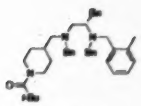

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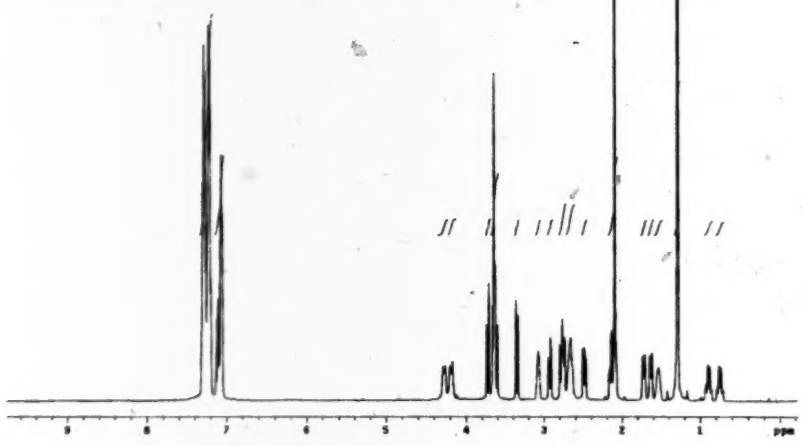

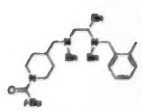

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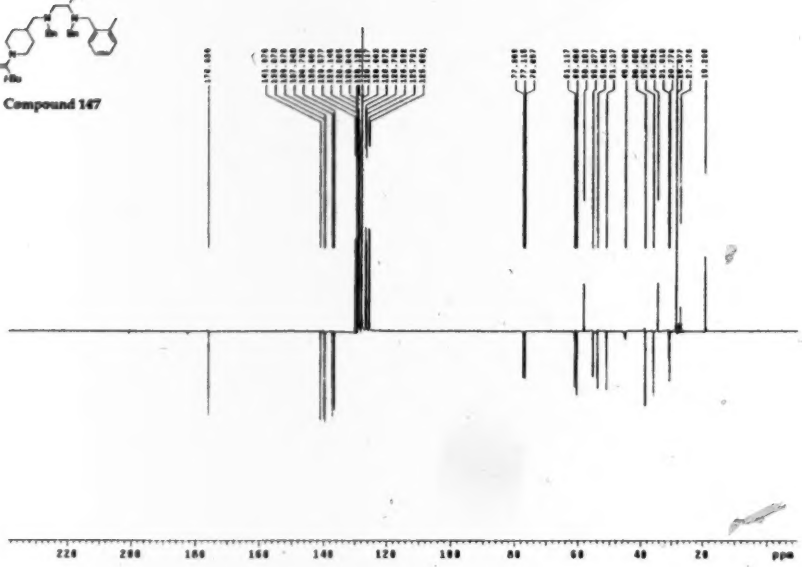
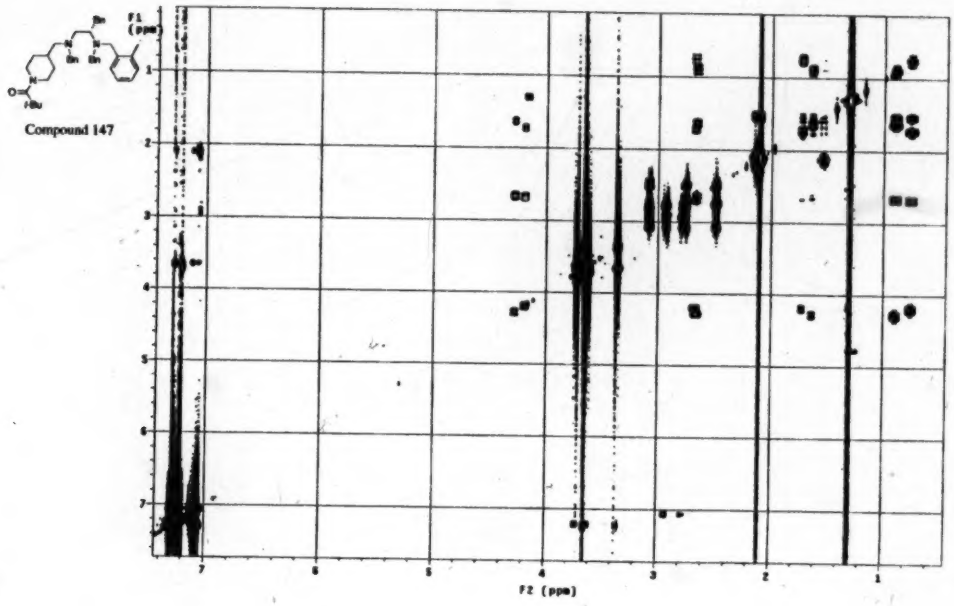
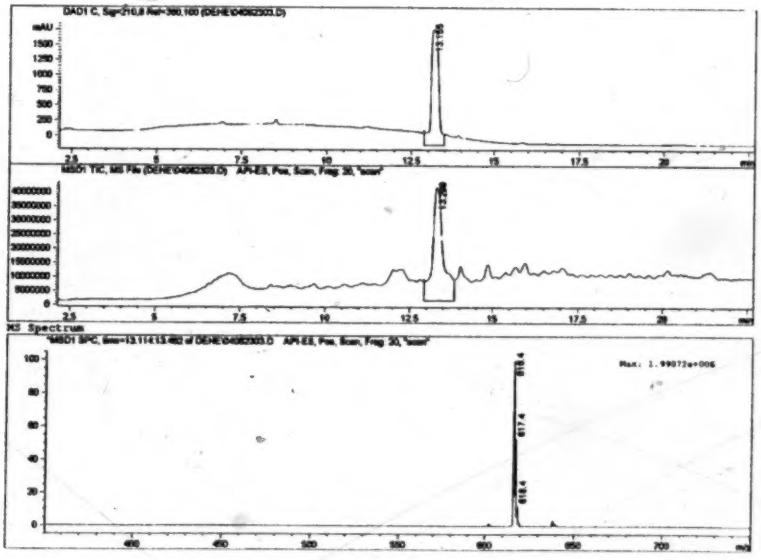
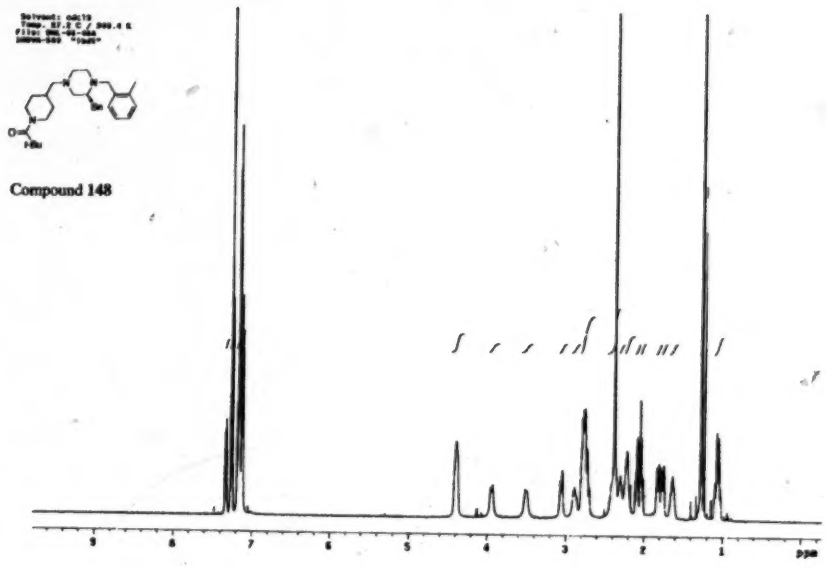




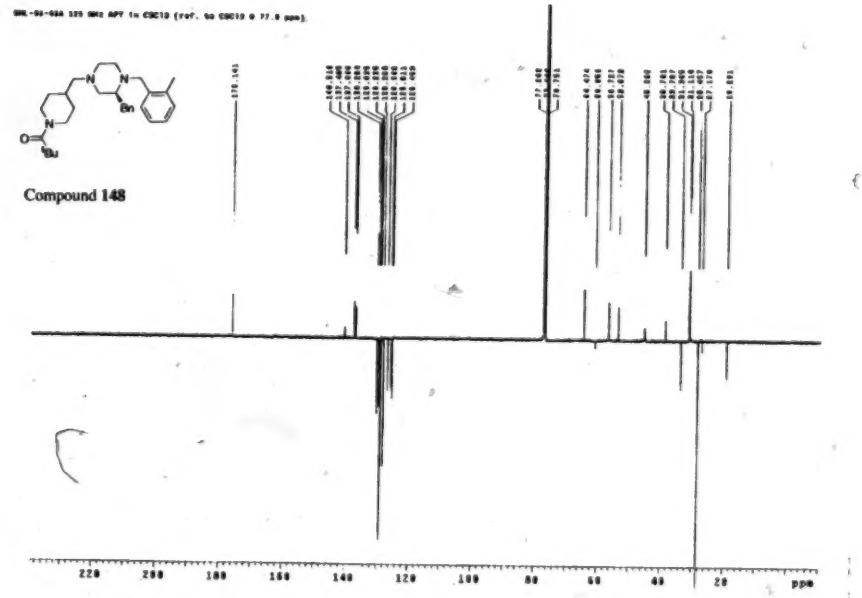
Compound 147

Compound **147**





Compound **148**



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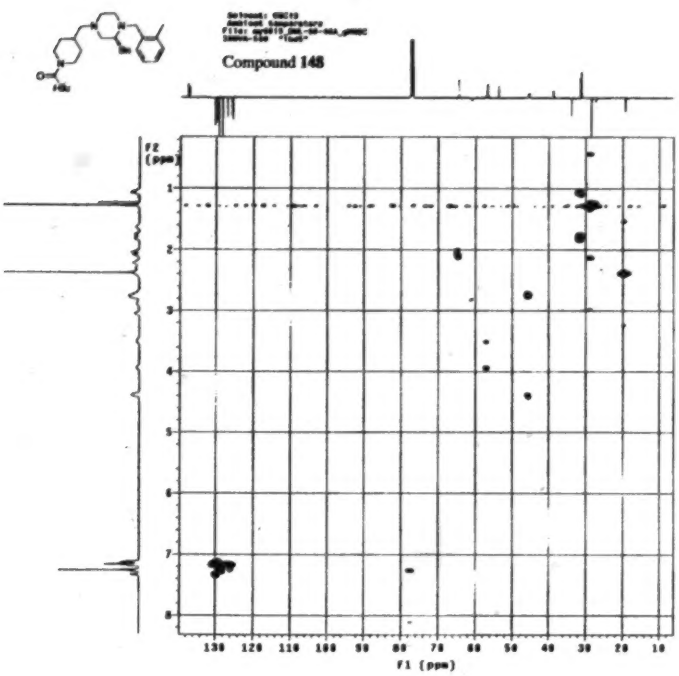
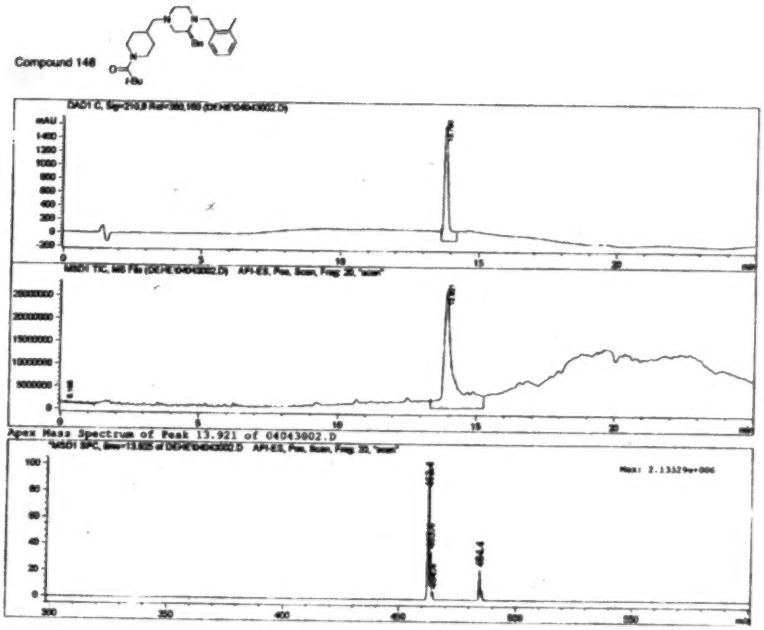
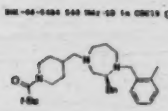
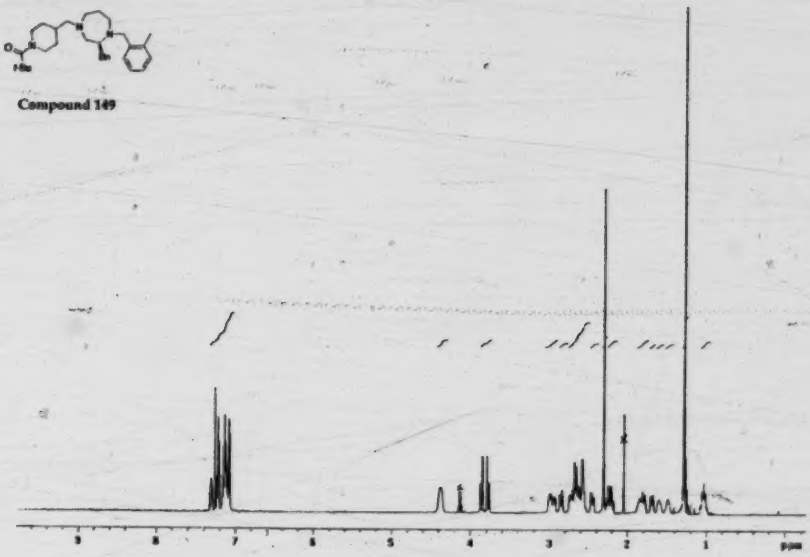
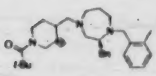

Compound 148
f2 (ppm)
F1 (ppm)


Compound 148
Apex Mass Spectrum of Peak 13.921 of 04043002.D

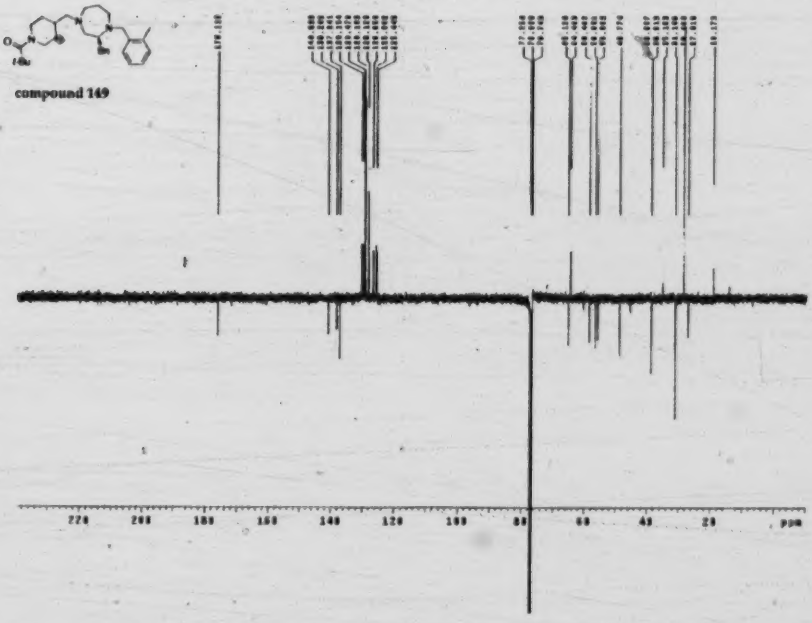


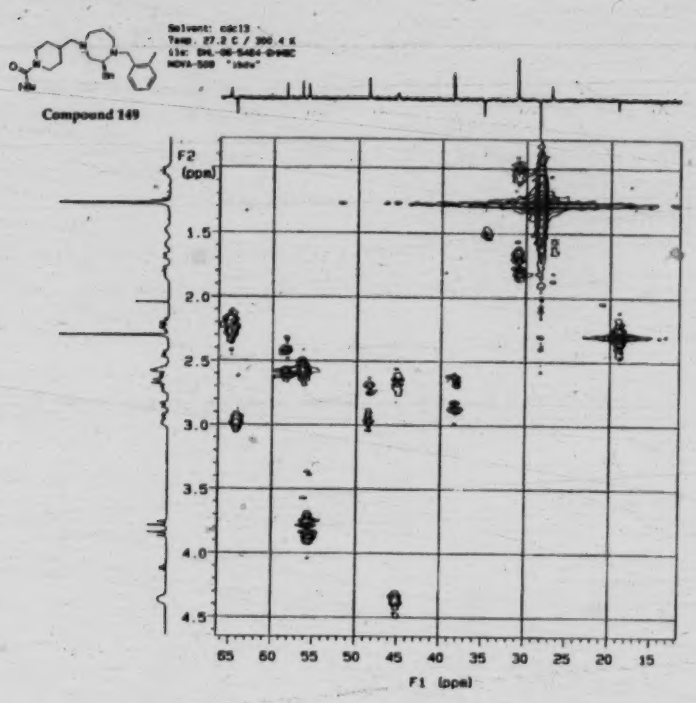
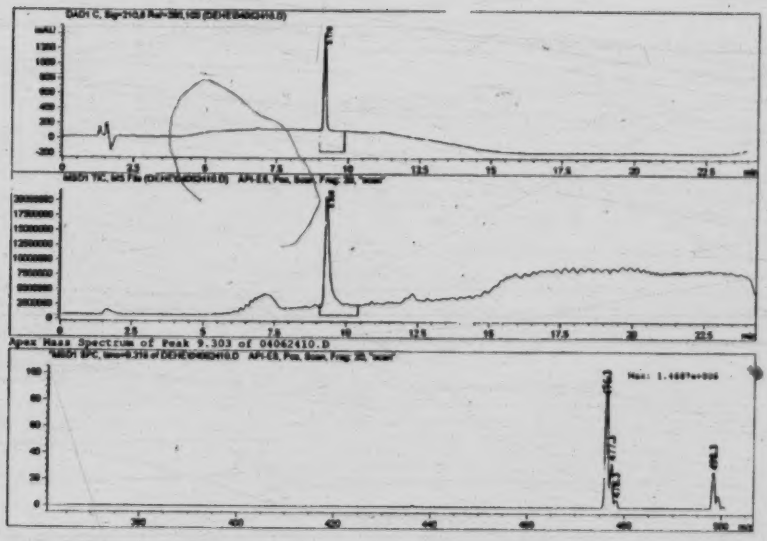
Compound 149





compound 149





Compound 149